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=> s l1 and CH2 domain
L2          593 L1 AND CH2 DOMAIN

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L8            0 L6 AND 235

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L9           11 DUP REMOVE L6 (9 DUPLICATES REMOVED)

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L10           3 L9 AND PD<19980508

=> d l10 1-3 cbib abs
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L10 ANSWER 1 OF 3 MEDLINE on STN
1999007292. PubMed ID: 9789066. Homogeneous immunoconjugates for boron neutron-capture therapy: design, synthesis, and preliminary characterization. Guan L; Wims L A; Kane R R; Smuckler M B; Morrison S L; Hawthorne M F. (Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095, USA.) Proceedings of the National Academy of Sciences of the United States of America, (1998 Oct 27) Vol. 95, No. 22, pp. 13206-10. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The application of immunoprotein-based targeting strategies to the boron neutron-capture therapy of cancer poses an exceptional challenge, because viable boron neutron-capture therapy by this method will require the efficient delivery of 10(3) boron-10 atoms by each antigen-binding protein. Our recent investigations in this area have been focused on the development of efficient methods for the assembly of homogeneous immunoprotein conjugates containing the requisite boron load. In this regard, engineered immunoproteins fitted with unique, exposed cysteine residues provide attractive vehicles for site-specific modification. Additionally, homogeneous oligomeric boron-rich phosphodiesters (oligophosphates) have been identified as promising conjugation reagents. The coupling of two such boron-rich oligophosphates to sulfhydryls introduced to the CH2 domain of a chimeric IgG3 has been demonstrated. The resulting boron-rich immunoconjugates are formed efficiently, are readily purified, and have promising in vitro and in vivo characteristics. Encouragingly, these studies showed subtle differences in the properties of the conjugates derived from the two oligophosphate molecules studied, providing a basis for the application of rational design to future work. Such subtle details would not have been as readily discernible in heterogeneous conjugates, thus validating the rigorous experimental design employed here.

L10 ANSWER 2 OF 3 MEDLINE on STN
92232133. PubMed ID: 1567557. Mapping rheumatoid factor binding sites using genetically engineered, chimeric IgG antibodies. Bonagura V R; Artandi S E; Agostino N; Tao M H; Morrison S L. (Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, NY 10032.) DNA and cell biology, (1992 Apr) Vol. 11, No. 3, pp. 245-52. Journal code: 9004522. ISSN: 1044-5498. Pub. country: United States. Language: English.

AB We are using chimeric IgG antibodies consisting of murine variable regions joined to human constant regions as rheumatoid factor (RF) binding substrates to localize and map IgM RF binding sites on IgG. Using chimeric antibodies in a modified RF ELISA, we showed that RFs from rheumatoid arthritis (RA) and Waldenstrom's macroglobulinemia (WMac) patients differ in their binding specificities for IgG3, although some of these RFs share common specificity for IgG1, IgG2, and IgG4. By shuffling constant region domains between IgG3 and IgG4, we showed that sequence variation in the CH3 domain is responsible for WMac-derived RF differentiation of IgG3 and IgG4. By making site-directed mutations in the wild-type IgG3 or IgG4 human gamma constant genes, we showed that His-435 is an essential residue in RF binding to IgG for most WMac RFs. The allotypic polymorphism in IgG3 at 436 is not responsible for differences in previous reports of high-frequency IgG3 binding by WMac RFs. A amino acid loop in the CH2 domain of IgG4 proximal to the CH2-CH3 interface is important in WMac RF binding to IgG; a more distal CH2 loop in CH2 has a more variable effect on WMac RF binding. To evaluate the contribution of the N-linked carbohydrate moiety at Asn-297 to RF binding sites on IgG, we measured RF binding to aglycosylated IgG antibodies produced by mutating the glycosylation signal Asn-297 to another amino acid. Of all

four IgG subclasses, only aglycosylated IgG3 was a better RF binding substrate than its glycosylated subclass counterpart.(ABSTRACT TRUNCATED AT 250 WORDS)

L10 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2008 ACS on STN
1990:476476 Document No. 113:76476 Original Reference No. 113:12937a,12940a
Antibodies having modified carbohydrate content and methods of preparation and use. Morrison, Sherie L.; Oi, Vernon T.; Hinton, Paul R. (Columbia University, USA; Becton, Dickinson and Co.). Eur. Pat. Appl. EP 359096 A1 19900321, 23 pp. DESIGNATED STATES: R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1989-116368 19890905. PRIORITY: US 1988-244744 19880915.
AB A method of altering the affinity of an antibody for the antigen to which it is directed comprises introducing into the variable region of the antibody a carbohydrate recognition site under conditions such that a carbohydrate binds to the site. The carbohydrate content is also modified by deleting from a constant region of the antibody a carbohydrate recognition site which naturally occurs in the constant region. The antibodies can be labeled, attached to a solid support, or conjugated with therapeutic ligands for use in anal., affinity chromatog., and therapy. The carbohydrate site in the CH2 domain of human IgG subclasses was deleted by site-directed mutagenesis of the DNA encoding the IgGs. The resultant antibodies had decreased ability to bind Fc receptors and to activate complement.

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L11 66 L2 AND SUBSTITUTION

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L12 30 L11 AND PD<19980508

=> s l12 and 231-340
L13 0 L12 AND 231-340

=> s l12 and reduced complement dependent lysis
L14 0 L12 AND REDUCED COMPLEMENT DEPENDENT LYSIS

=> s l12 and Fc receptor
L15 7 L12 AND FC RECEPTOR

=> d l15 1-7 cbib abs

L15 ANSWER 1 OF 7 MEDLINE on STN
89078461. PubMed ID: 3060362. Complement activation is not required for IgG-mediated suppression of the antibody response. Heyman B; Wiersma E; Nose M. (Department of Immunology, Uppsala University, Sweden.) European journal of immunology, (1988 Nov) Vol. 18, No. 11, pp. 1739-43. Journal code: 1273201. ISSN: 0014-2980. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.
AB Feedback suppression of the antibody response by IgG is known to be dependent on intact Fc regions. However, it is not clear which of the Fc-mediated effector functions is required. In the present report we have studied whether ability or inability of the IgG antibodies to activate the complement system was of consequence for their immunosuppressive effect. First, a monoclonal IgG1-anti-2,4,6-trinitrophenyl (TNP) antibody, unable to activate complement via the classical or alternate pathway, was shown to be able to inhibit more

than 90% of the in vivo sheep erythrocyte-specific antibody response in mice when TNP coupled to sheep erythrocytes was used as antigen. Second, we investigated the immunosuppressive ability of a non-complement-activating mutant IgG2a-anti-TNP monoclonal antibody. The mutant differs from the wild type by a single amino acid substitution in the CH2 domain leading to inability to fix complement factor C1q. However, the mutant has the same affinity for antigen and the same Fc receptor -binding capacity as the wild type antibody. It is demonstrated that the mutant was as efficient as the wild type antibody in inhibiting an in vitro antibody response to TNP-coupled sheep erythrocytes. These findings confirm the non-determinant specificity and Fc dependence of IgG-mediated suppression, and show that the Fc-mediated effector mechanism is independent of complement activation. The results instead suggest binding to Fc receptors as a necessary step in feedback immunosuppression and favor inactivation of B cells by cross-linking of Fc and antigen receptors on their surface rather than elimination of antigen by complement-dependent phagocytosis as the effector mechanism.

L15 ANSWER 2 OF 7 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN

1989011581 EMBASE Complement activation is not required for IgG-mediated suppression of the antibody response.

Heyman, B.; Wiersma, E.; Nose, M.. Department of Immunology, Uppsala University, Biomedical Center, S-75123 Uppsala, Sweden.

European Journal of Immunology Vol. 18, No. 11, pp. 1739-1743 1988.
ISSN: 0014-2980. CODEN: EJIMAF
Pub. Country: Germany. Language: English. Summary Language: English.
Entered STN: 911212. Last Updated on STN: 911212

AB Feedback suppression of the antibody response by IgG is known to be dependent on intact Fc regions. However, it is not clear which of the Fc-mediated effector functions is required. In the present report we have studied whether ability or inability of the IgG antibodies to activate the complement system was of consequence for their immunosuppressive effect. First, a monoclonal IgG(1)-anti-2,4,6-trinitrophenyl (TNP) antibody, unable to activate complement via the classical or alternate pathway, was shown to be able to inhibit more than 90% of the in vivo sheep erythrocyte-specific antibody response in mice when TNP coupled to sheep erythrocytes was used as antigen. Second, we investigated the immunosuppressive ability of a non-complement-activating mutant IgG(2a)-anti-TNP monoclonal antibody. The mutant differs from the wild type by a single amino acid substitution in the CH2 domain leading to inability to fix complement factor C1q. However, the mutant has the same affinity for antigen and the same Fc receptor -binding capacity as the wild type antibody. It is demonstrated that the mutant was as efficient as the wild type antibody in inhibiting an in vitro antibody response to TNP-coupled sheep erythrocytes. These findings confirm the non-determinant specificity and Fc dependence of IgG-mediated suppression, and show that the Fc-mediated effector mechanism is independent of complement activation. The results instead suggest binding to Fc receptors as a necessary step in feedback immunosuppression and favor inactivation of B cells by cross-linking of Fc and antigen receptors on their surface rather than elimination of antigen by complement-dependent phagocytosis as the effector mechanism.

L15 ANSWER 3 OF 7 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN

1989:125014 Document No.: PREV198987059667; BA87:59667. COMPLEMENT ACTIVATION

IS NOT REQUIRED FOR IGG-MEDIATED SUPPRESSION OF THE ANTIBODY RESPONSE. HEYMAN B [Reprint author]; WIERSMA E; NOSE M. DEP IMMUNOL, BOX 582, BMC, S-751 23 UPPSALA, SWED. European Journal of Immunology, (1988) Vol. 18, No. 11, pp. 1739-1744.
CODEN: EJIMAF. ISSN: 0014-2980. Language: ENGLISH.

AB Feedback suppression of the antibody response by IgG is known to be dependent on intact Fc regions. However, it is not clear which of the Fc-mediated effector functions is required. In the present report we have studied whether ability or inability of the IgG antibodies to activate the complement system was of consequence for their immunosuppressive effect. First, a monoclonal IgG1-anti-2,4,6-trinitrophenyl (TNP) antibody, unable to activate complement via the classical or alternate pathway, was shown to be able to inhibit more than 90% of the in vivo sheep erythrocyte-specific antibody response in mice when TNP coupled to sheep erythrocytes was used as antigen. Second, we investigated the immunosuppressive ability of a non-complement-activating mutant IgG2a-anti-TNP monoclonal antibody. The mutant differs from the wild type by a single amino acid substitution in the CH2 domain leading to inability to fix complement factor C1q. However, the mutant has the same affinity for antigen and the same Fc receptor -binding capacity as the wild type antibody. It is demonstrated that the mutant was as efficient as the wild type antibody in inhibiting an in vitro antibody response to TNP-coupled sheep erythrocytes. These findings confirm the non-determinant specificity and Fc dependence of IgG-mediated suppression, and show that the Fc-mediated effector mechanism is independent of complement activation. The results instead suggest binding to Fc receptors as a necessary step in feedback immunosuppression and favor inactivation of B cells by cross-linking of Fc antigen receptors on their surface rather than elimination of antigen by complement-dependent phagocytosis as the effector mechanism.

L15 ANSWER 4 OF 7 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

1991:313325 The Genuine Article (R) Number: FN859. THE BINDING-AFFINITY OF HUMAN-IGG FOR ITS HIGH-AFFINITY FC RECEPTOR IS DETERMINED BY MULTIPLE AMINO-ACIDS IN THE CH2 DOMAIN AND IS MODULATED BY THE HINGE REGION. CANFIELD S M (Reprint); MORRISON S L. COLUMBIA UNIV COLL PHYS & SURG, DEPT MICROBIOL, NEW YORK, NY 10032 (Reprint); UNIV CALIF LOS ANGELES, DEPT MICROBIOL & MOLEC GENET, LOS ANGELES, CA 90024; UNIV CALIF LOS ANGELES, INST MOLEC BIOL, LOS ANGELES, CA 90024. JOURNAL OF EXPERIMENTAL MEDICINE (1 JUN 1991) Vol. 173, No. 6, pp. 1483-1491. ISSN: 0022-1007. Publisher: ROCKEFELLER UNIV PRESS, 222 E 70TH STREET, NEW YORK, NY 10021. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A family of chimeric immunoglobulins (Igs) bearing the murine variable region directed against the hapten dansyl linked to human IgG1, -2, -3, and -4 has been characterized with respect to binding to the human high affinity Fc-gamma-receptor, Fc-gamma-R(I). Chimeric IgG1 and -3 have the highest affinity association (K(a) = 10(9) M-1), IgG4 is 10-fold reduced from this level, and IgG2 displays no detectable binding. A series of genetic manipulations was undertaken in which domains from the strongly binding subclass IgG3 were exchanged with domains from the nonbinding subclass IgG2. The subclass of the C(H)2 domain was found to be critical for determining IgG receptor affinity. In addition, the hinge region was found to modulate the affinity of the IgG for Fc-gamma-R(I), possibly by determining accessibility of Fc-gamma-R(I) to the binding site on Fc. A series of amino acid substitutions were engineered into the C(H)2 domain of IgG3 and IgG4 at sites considered potentially important to Fc receptor binding based on

homology comparisons of binding and nonbinding IgG subclasses. Characterization of these mutants has revealed the importance for Fc-gamma-R(I) association of two regions of the genetic C(H)2 domain separated in primary structure by nearly 100 residues. The first of these is the hinge-link or lower hinge region, in which two residues, Leu(234) and Leu(235) in IgG1 and -3, are critical to high affinity binding. Substitution at either of these sites reduces the IgG association constant by 10-100-fold. The second region that appears to contribute to receptor binding is in a hinge-proximal bend between two beta-strands within the C(H)2 domain, specifically, Pro(331) in IgG1 and -3. As a result of beta-sheet formation within this domain, this residue lies within 11 angstrom of the hinge-link region. Substitution at this site reduces the Fc receptor association constant by 10-fold.

L15 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2008 ACS on STN
1991:677551 Document No. 115:277551 Original Reference No. 115:47125a,47128a
Identification of the Fcγ receptor class I binding site in human IgG through the use of recombinant IgG1/IgG2 hybrid and point-mutated antibodies. Chappel, M. Suzanne; Isenman, David E.; Everett, Margaret; Xu, Yuan Yuan; Dorrington, Keith J.; Klein, Michael H. (Dep. Immunol., Univ. Toronto, Toronto, ON, M5S 1A8, Can.). Proceedings of the National Academy of Sciences of the United States of America, 88(20), 9036-40 (English) 1991. CODEN: PNASA6. ISSN: 0027-8424.
AB To characterize the region on human IgG1 responsible for its high-affinity interaction with the human Fcγ receptor class I (FcγRI), the authors have analyzed the binding properties of a series of genetically engineered chimeric antidinitrophenyl antibodies with identical murine antibody combining sites and hybrid IgG1/IgG2 human constant (C) regions. In addition, a panel of reciprocally point-mutated IgG1 and IgG2 chimeric antibodies was investigated to identify the amino acid residues that confer cytophilic properties to human IgG1. The data unambiguously indicate that cytophilic activity of IgG1 is an intrinsic property of its heavy-chain C region 2 (CH2) domain. The entire sequence spanning residues 234-237 (LLGG) is required to restore full binding activity to IgG2 and IgG4 and individual amino acid substitutions failed to render IgG2 active. Nevertheless, the reciprocal single point mutations in IgG1 either lowered its activity or abolished it completely. An IgG2 antibody containing the entire ELLGGP sequence (residues 233-238) was more active than wild-type IgG1. Thus, in addition to the primary contact site identified in the N terminus of the γ1 CH2 domain, secondary sites involving residues from the C-terminal half of the domain may also contribute to the IgG1-FcγRI interaction.

L15 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2008 ACS on STN
1989:207251 Document No. 110:207251 Original Reference No. 110:34286h,34287a
Altered antibodies having altered effector functions and their preparation. Winter, Gregory Paul; Duncan, Alexander Robert; Burton, Dennis Raymond (Medical Research Council, UK). PCT Int. Appl. WO 8807089 A1 19880922, 42 pp. DESIGNATED STATES: W: AU, GB, JP, US; RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1988-GB211 19880318. PRIORITY: GB 1987-6425 19870318; GB 1987-18897 19870810; GB 1987-28042 19871201.
AB Antibodies (Ab) with altered binding affinity for effectors such as the C1q component of the complement system are prepared by replacing amino acid residues of the CH2 region using genetic engineering techniques. Human Cγ3 genes having a mutation, i.e. 234-leucine to alanine, 235-leuciner to glutamine, 236-glycine to alanine, and 237-glycine to alanine, resp., were constructed and cloned into expression vector pSBgpt after linking with the gene encoding the variable domain of

the B18 antibody (Ab). The binding affinity I50 (the concentration of IgG3 at which the fractional binding of 125I-labeled pooled human IgG is 0.5) to Fc γ R1 receptor on U937 cells of the recombinant mutants, i.e. [234-Ala]-IgG3, [235-Glu]-IgG3, [236-Ala]-IgG3, and [237-Ala]-IgG3 were 4 + 10-8, >10-6, 3 + 10-8, and 3 + 10-7M, resp., vs. 10-8M of the control using the wild-type IgG.

L15 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2008 ACS on STN
1989:73542 Document No. 110:73542 Original Reference No. 110:12123a,12126a
Complement activation is not required for IgG-mediated suppression of the antibody response. Heyman, Birgitta; Wiersma, Erik; Nose, Masato (Biomed. Cent., Uppsala, Uppsala, S-75123, Swed.). European Journal of Immunology, 18(11), 1739-43 (English) 1988. CODEN: EJIMAF. ISSN: 0014-2980.
AB The authors studied whether the ability or inability of IgG antibodies to activate the complement system was of consequence for their immunosuppressive effect on the antibody response. A monoclonal IgG1-anti-2,4,6-trinitrophenyl (TNP) antibody, unable to activate complement via the classical or alternate pathway, inhibited >90% of the in vivo sheep erythrocyte-specific antibody response in mice when TNP coupled to sheep erythrocytes was used as antigen. The immunosuppressive ability was investigated of a non-complement-activating mutant IgG2a-anti-TNP monoclonal antibody. The mutant differs from the wild type by a single amino acid substitution in the CH2 domain leading to inability to fix complement factor C1q. However, the mutant has the same affinity for antigen and the same Fc receptor-binding capacity as the wild type antibody. The mutant was as efficient as the wild type antibody in inhibiting an in vitro antibody response to TNP-coupled sheep erythrocytes. These findings confirm the non-determinant specificity and Fc-dependence of IgG-mediated suppression, and show that the Fc-mediated effector mechanism is independent of complement activation. The results suggest binding to Fc receptors as a necessary step in feedback immunosuppression and favor inactivation of B cells by crosslinking of Fc and antigen receptors on their surface rather than elimination of antigen by complement-dependent phagocytosis as the effector mechanism.

=> s l12 and complement
L16 13 L12 AND COMPLEMENT

=> d l16 1-13 cbib abs

L16 ANSWER 1 OF 13 MEDLINE on STN
95309977. PubMed ID: 7540592. Activation of effector functions by immune complexes of mouse IgG2a with isotype-specific autoantibodies. Rajnavolgyi E; Fazekas G; Lund J; Daeron M; Teillaud J L; Jefferis R; Fridman W H; Gergely J. (Department of Immunology, L. Eotvos University, God, Hungary.) Immunology, (1995 Apr) Vol. 84, No. 4, pp. 645-52. Journal code: 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Analysis of five monoclonal autoantibodies, rheumatoid factors produced by hybridomas generated from spleen cells of BALB/c mice repeatedly infected with A/PR/8/34 human influenza A virus, revealed that they recognized distinct but spatially related epitopes. The differing isoallotypic specificity of the IgM and IgA monoclonal antibodies correlated with the presence of Ile258 and Ala305, respectively. Although these data suggest that the epitopes recognized are within the CH2 domain, all antibodies failed to inhibit IgG antigen reactivity with Staphylococcus aureus protein A (SpA), C1q, mouse C3,

human Fc gamma RI or mouse Fc gamma RII, activities known to be predominantly determined by CH2 domain structures. Reactivity of the IgA antibody, Z34, with IgG2b allowed further specificity studies using a panel of 26 mutant IgG2b proteins, each having single amino acid replacements over the surface of the CH2 domain. The only substitution that affected Z34 reactivity was Asn/Ala297, which destroyed the glycosylation sequon, resulting in secretion of an aglycosylated IgG molecule. The epitope recognized by Z34 therefore seems to be located outside of the Fc gamma R and C1q binding sites, but to be dependent on the presence of carbohydrate for expression. In contrast to the binding studies, complement activation by aggregated IgG2a, through classical or alternative pathways, was inhibited by the presence of autoantibodies. The functional significance of isotype-specific autoantibody in immune regulation is discussed.

L16 ANSWER 2 OF 13 MEDLINE on STN
94148845. PubMed ID: 8106388. Residue at position 331 in the IgG1 and IgG4 CH2 domains contributes to their differential ability to bind and activate complement. Xu Y; Oomen R; Klein M H. (Department of Immunology, University of Toronto, Ontario, Canada.) The Journal of biological chemistry, (1994 Feb 4) Vol. 269, No. 5, pp. 3469-74. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB A conserved proline residue is found at position 331 in the CH2 domains of human IgG subclasses which fix complement. This residue is replaced by a serine in IgG4 which is inactive. To determine the role of residue 331 in the differential ability of human IgGs to activate the complement cascade, a pair of genetically engineered anti-dinitrophenol IgG1 and IgG4 antibodies with reciprocal mutations at position 331 were tested for their hemolytic activity as well as for their ability to bind C1q, activate C1 and cleave C4. The IgG1 Ser331 mutant was virtually unable to mediate the lysis of trinitrobenzene-sulfonic acid-derivatized sheep red blood cells as a result of a marked defect in C1q binding activity. In contrast, the substitution of Pro for Ser331 in IgG4 bestowed partial hemolytic activity (40%) to the IgG4 Pro331 variant. Under low ionic strength conditions, this mutant was found to be approximately 50 and 75% as active as wild-type IgG1 in the C1q binding and C4b deposition assays, respectively. These results indicate that residue Pro331, which folds into close proximity to a previously identified C1q binding motif (Duncan, A. R., and Winter, G. (1988) Nature 332, 738-740), contributes to the architecture of the IgG1 C1q binding site and that its replacement by a serine residue in IgG4 is largely responsible for the functional inactivity of this isotype.

L16 ANSWER 3 OF 13 MEDLINE on STN
89078461. PubMed ID: 3060362. Complement activation is not required for IgG-mediated suppression of the antibody response. Heyman B; Wiersma E; Nose M. (Department of Immunology, Uppsala University, Sweden.) European journal of immunology, (1988 Nov) Vol. 18, No. 11, pp. 1739-43. Journal code: 1273201. ISSN: 0014-2980. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.
AB Feedback suppression of the antibody response by IgG is known to be dependent on intact Fc regions. However, it is not clear which of the Fc-mediated effector functions is required. In the present report we have studied whether ability or inability of the IgG antibodies to activate the complement system was of consequence for their immunosuppressive effect. First, a monoclonal IgG1-anti-2,4,6-trinitrophenyl (TNP) antibody, unable to activate

complement via the classical or alternate pathway, was shown to be able to inhibit more than 90% of the in vivo sheep erythrocyte-specific antibody response in mice when TNP coupled to sheep erythrocytes was used as antigen. Second, we investigated the immunosuppressive ability of a non-complement-activating mutant IgG2a-anti-TNP monoclonal antibody. The mutant differs from the wild type by a single amino acid substitution in the CH2 domain leading to inability to fix complement factor C1q. However, the mutant has the same affinity for antigen and the same Fc receptor-binding capacity as the wild type antibody. It is demonstrated that the mutant was as efficient as the wild type antibody in inhibiting an in vitro antibody response to TNP-coupled sheep erythrocytes. These findings confirm the non-determinant specificity and Fc dependence of IgG-mediated suppression, and show that the Fc-mediated effector mechanism is independent of complement activation. The results instead suggest binding to Fc receptors as a necessary step in feedback immunosuppression and favor inactivation of B cells by cross-linking of Fc and antigen receptors on their surface rather than elimination of antigen by complement -dependent phagocytosis as the effector mechanism.

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1995118404 EMBASE Activation of effector functions by immune complexes of mouse IgG2a with isotype-specific autoantibodies.

Rajnavolgyi, E., Dr. (correspondence); Fazekas, G.; Lund, J.; Daeron, M.; Teillaud, J.-L.; Jefferis, R.; Fridman, W.H.; Gergely, J.. Department of Immunology, L. Eotvos University, Javorka S.u., H-14 2131 God, Hungary.

Immunology Vol. 84, No. 4, pp. 645-652 1995.

ISSN: 0019-2805. CODEN: IMMUAM

Pub. Country: United Kingdom. Language: English. Summary Language: English.

Entered STN: 950516. Last Updated on STN: 950516

AB Analysis of five monoclonal autoantibodies, rheumatoid factors produced by hybridomas generated from spleen cells of BALB/c mice repeatedly infected with A/PR/8/34 human influenza A virus, revealed that they recognized distinct but spatially related epitopes. The differing isoallotypic specificity of the IgM and IgA monoclonal antibodies correlated with the presence of Ile258 and Ala305, respectively. Although these data suggest that the epitopes recognized are within the CH2 domain, all antibodies failed to inhibit IgG antigen reactivity with Staphylococcus aureus protein A (SpA), C1q, mouse C3, human FcγRI or mouse FcγRII, activities known to be predominantly determined by CH2 domain structures. Reactivity of the IgA antibody, Z34, with IgG2b allowed further specificity studies using a panel of 26 mutant IgG2b proteins, each having single amino acid replacements over the surface of the CH2 domain. The only substitution that affected Z34 reactivity was Asn/Ala297, which destroyed the glycosylation sequon, resulting in secretion of an aglycosylated IgG molecule. The epitope recognized by Z34 therefore seems to be located outside of the FcγR and C1q binding sites, but to be dependent on the presence of carbohydrate for expression. In contrast to the binding studies, complement activation by aggregated IgG2a, through classical or alternative pathways, was inhibited by the presence of autoantibodies. The functional significance of isotype-specific autoantibody in immune regulation is discussed.

L16 ANSWER 5 OF 13 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN

1989011581 EMBASE Complement activation is not required for

IgG-mediated suppression of the antibody response.
Heyman, B.; Wiersma, E.; Nose, M.. Department of Immunology, Uppsala University, Biomedical Center, S-75123 Uppsala, Sweden.
European Journal of Immunology Vol. 18, No. 11, pp. 1739-1743
1988.
ISSN: 0014-2980. CODEN: EJIMAF
Pub. Country: Germany. Language: English. Summary Language: English.
Entered STN: 911212. Last Updated on STN: 911212

AB Feedback suppression of the antibody response by IgG is known to be dependent on intact Fc regions. However, it is not clear which of the Fc-mediated effector functions is required. In the present report we have studied whether ability or inability of the IgG antibodies to activate the complement system was of consequence for their immunosuppressive effect. First, a monoclonal IgG(1)-anti-2,4,6-trinitrophenyl (TNP) antibody, unable to activate complement via the classical or alternate pathway, was shown to be able to inhibit more than 90% of the in vivo sheep erythrocyte-specific antibody response in mice when TNP coupled to sheep erythrocytes was used as antigen. Second, we investigated the immunosuppressive ability of a non-complement-activating mutant IgG(2a)-anti-TNP monoclonal antibody. The mutant differs from the wild type by a single amino acid substitution in the CH2 domain leading to inability to fix complement factor Clq. However, the mutant has the same affinity for antigen and the same Fc receptor-binding capacity as the wild type antibody. It is demonstrated that the mutant was as efficient as the wild type antibody in inhibiting an in vitro antibody response to TNP-coupled sheep erythrocytes. These findings confirm the non-determinant specificity and Fc dependence of IgG-mediated suppression, and show that the Fc-mediated effector mechanism is independent of complement activation. The results instead suggest binding to Fc receptors as a necessary step in feedback immunosuppression and favor inactivation of B cells by cross-linking of Fc and antigen receptors on their surface rather than elimination of antigen by complement -dependent phagocytosis as the effector mechanism.

L16 ANSWER 6 OF 13 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
1995:253578 Document No.: PREV199598267878. Activation of effector functions by immune complexes of mouse IgG2a with isotype-specific autoantibodies.
Rajnavolgyi, E. [Reprint author]; Fazekas, G.; Lund, J.; Daeron, M.; Teillaud, J.-L.; Jefferis, R.; Fridman, W. H.; Gergely, J.. Dep. Immunol., L. Eotvos Univ., Javorka S. u., H-14 2131 God, Hungary. Immunology, (1995) Vol. 84, No. 4, pp. 645-652.
CODEN: IMMUAM. ISSN: 0019-2805. Language: English.

AB Analysis of five monoclonal autoantibodies, rheumatoid factors produced by hybridomas generated from spleen cells of BALB/c mice repeatedly infected with A/PR/8/34 human influenza A virus, revealed that they recognized distinct but spatially related epitopes. The differing isoallotypic specificity of the IgM and IgA monoclonal antibodies correlated with the presence of Ile258 and Ala305, respectively. Although these data suggest that the epitopes recognized are within the CH2 domain, all antibodies failed to inhibit IgG antigen reactivity with Staphylococcus aureus protein A (SpA), C1q, mouse C3, human Fc-gamma-RI or mouse Fc-gamma-RII, activities known to be predominantly determined by CH2 domain structures. Reactivity of the IgA antibody, Z34, with IgG2b allowed further specificity studies using a panel of 26 mutant IgG2b proteins, each having single amino acid replacements over the surface of the CH2 domain. The only substitution that affected Z34 reactivity was Asn/Ala297, which destroyed the glycosylation sequon, resulting in secretion of an aglycosylated IgG molecule. The epitope

recognized by Z34 therefore seems to be located outside of the Fc-gamma-R and C1q binding sites, but to be dependent on the presence of carbohydrate for expression. In contrast to the binding studies, complement activation by aggregated IgG2a, through classical or alternative pathways, was inhibited by the presence of autoantibodies. The functional significance of isotype-specific autoantibody in immune regulation is discussed.

L16 ANSWER 7 OF 13 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
1989:125014 Document No.: PREV198987059667; BA87:59667. COMPLEMENT ACTIVATION IS NOT REQUIRED FOR IGG-MEDIATED SUPPRESSION OF THE ANTIBODY RESPONSE. HEYMAN B [Reprint author]; WIERSMA E; NOSE M. DEP IMMUNOL, BOX 582, BMC, S-751 23 UPPSALA, SWED. European Journal of Immunology, (1988) Vol. 18, No. 11, pp. 1739-1744. CODEN: EJIMAF. ISSN: 0014-2980. Language: ENGLISH.
AB Feedback suppression of the antibody response by IgG is known to be dependent on intact Fc regions. However, it is not clear which of the Fc-mediated effector functions is required. In the present report we have studied whether ability or inability of the IgG antibodies to activate the complement system was of consequence for their immunosuppressive effect. First, a monoclonal IgG1-anti-2,4,6-trinitrophenyl (TNP) antibody, unable to activate complement via the classical or alternate pathway, was shown to be able to inhibit more than 90% of the in vivo sheep erythrocyte-specific antibody response in mice when TNP coupled to sheep erythrocytes was used as antigen. Second, we investigated the immunosuppressive ability of a non-complement-activating mutant IgG2a-anti-TNP monoclonal antibody. The mutant differs from the wild type by a single amino acid substitution in the CH2 domain leading to inability to fix complement factor C1q. However, the mutant has the same affinity for antigen and the same Fc receptor-binding capacity as the wild type antibody. It is demonstrated that the mutant was as efficient as the wild type antibody in inhibiting an in vitro antibody response to TNP-coupled sheep erythrocytes. These findings confirm the non-determinant specificity and Fc dependence of IgG-mediated suppression, and show that the Fc-mediated effector mechanism is independent of complement activation. The results instead suggest binding to Fc receptors as a necessary step in feedback immunosuppression and favor inactivation of B cells by cross-linking of Fc antigen receptors on their surface rather than elimination of antigen by complement -dependent phagocytosis as the effector mechanism.

L16 ANSWER 8 OF 13 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
1995:284059 The Genuine Article (R) Number: QU531. ACTIVATION OF EFFECTOR FUNCTIONS BY IMMUNE-COMPLEXES OF MOUSE IGG2A WITH ISOTYPE-SPECIFIC AUTOANTIBODIES. RAJNAVOLGYI E (Reprint); FAZEKAS G; LUND J; DAERON M; TEILLAUD J L; JEFFERIS R; FRIDMAN W H; GERGELY J. LORAND EOTVOS UNIV, DEPT IMMUNOL, JAVORKA SU, H-142131 GODOLLO, HUNGARY (Reprint); UNIV BIRMINGHAM, SCH MED, DEPT IMMUNOL, BIRMINGHAM, W MIDLANDS, ENGLAND; INST CURIE, INSERM, U255, PARIS, FRANCE. IMMUNOLOGY (APR 1995) Vol. 84, No. 4, pp. 645-652. ISSN: 0019-2805. Publisher: BLACKWELL SCIENCE LTD, OSNEY MEAD, OXFORD, OXON, ENGLAND OX2 0EL. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Analysis of five monoclonal autoantibodies, rheumatoid factors produced by hybridomas generated from spleen cells of BALB/c mice repeatedly infected with A/PR/8/34 human influenza A virus, revealed that they recognized distinct but spatially related epitopes. The differing isoallotypic specificity of the IgM and IgA monoclonal antibodies correlated with the presence of Ile258 and Ala305, respectively. Although

these data suggest that the epitopes recognized are within the CH2 domain, all antibodies failed to inhibit Ige antigen reactivity with Staphylacoccus aureas protein A (SpA), Clq, mouse C3, human Fc gamma RI or mouse Fc gamma RII, activities known to be predominantly determined by CH2 domain structures. Reactivity of the IgA antibody, Z34, with IgG2b allowed further specificity studies using a panel of 26 mutant IgG2b proteins, each having single amino acid replacements over the surface of the CH2 domain. The only substitution that affected Z34 reactivity was Asn/Ala297, which destroyed the glycosylation sequon, resulting in secretion of an aglycosylated IgG molecule. The epitope recognized by Z34 therefore seems to be located outside of the Fc-IR and Clq binding sites, but to be dependent on the presence of carbohydrate for expression. In contrast to the binding studies, complement activation by aggregated IgG2a, through classical or alternative pathways, was inhibited by the presence of autoantibodies. The functional significance of isotype-specific autoantibody in immune regulation is discussed.

L16 ANSWER 9 OF 13 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

1994:70790 The Genuine Article (R) Number: MV631. RESIDUE AT POSITION-331 IN THE IGG1 AND IGG4 CH2 DOMAINS CONTRIBUTES TO THEIR DIFFERENTIAL ABILITY TO BIND AND ACTIVATE COMPLEMENT. XU Y (Reprint); OOMEN R; KLEIN M H. UNIV TORONTO, DEPT IMMUNOL, MED SCI BLDG, TORONTO M5S 1A8, ONTARIO, CANADA; CONNAUGHT CTR BIOTECHNOL RES, N YORK M2R 3T4, ON, CANADA; UNIV TORONTO, DEPT BIOCHEM, TORONTO M5S lA8, ONTARIO, CANADA. JOURNAL OF BIOLOGICAL CHEMISTRY (4 FEB 1994) Vol. 269, No. 5, pp. 3469-3474. ISSN: 0021-9258. Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A conserved proline residue is found at position 331 in the C(H)2 domains of human IgG subclasses which fix complement. This residue is replaced by a serine in IgG4 which is inactive. To determine the role of residue 331 in the differential ability of human IgGs to activate the complement cascade, a pair of genetically engineered anti-dinitrophenol IgG1 and IgG4 antibodies with reciprocal mutations at position 331 were tested for their hemolytic activity as well as for their ability to bind C1q, activate C1 and cleave C4. The IgG1 Ser331 mutant was virtually unable to mediate the lysis of trinitrobenzene-sulfonic acid-derivatized sheep red blood cells as a result of a marked defect in C1q binding activity. In contrast, the substitution of Pro for Ser331 in IgG4 bestowed partial hemolytic activity (40%) to the IgG4 Pro331 variant. Under low ionic strength conditions, this mutant was found to be approximately 50 and 75% as active as wild-type IgG1 in the C1q binding and C4b deposition assays, respectively. These results indicate that residue Pro''', which folds into close proximity to a previously identified C1q binding motif (Duncan, A. R., and Winter, G. (1988) Nature 332, 738-740), contributes to the architecture of the IgG1 C1q binding site and that its replacement by a serine residue in IgG4 is largely responsible for the functional inactivity of this isotype.

L16 ANSWER 10 OF 13 CAPLUS COPYRIGHT 2008 ACS on STN

1995:518071 Document No. 122:288749 Original Reference No. 122:52635a,52638a Activation of effector functions by immune complexes of mouse IgG2a with isotype-specific autoantibodies. Rajnavoelgyi, E.; Fazekas, G.; Lund, J.; Daeron, M.; Teillaud, J.-L.; Jefferis, R.; Fridman, W. H.; Gergely, J. (Dep. Immunology, L. Eoetvoes Univ., Goed, Hung.). Immunology, 84(4), 645-52 (English) 1995. CODEN: IMMUAM. ISSN: 0019-2805.

Publisher: Blackwell.

AB Anal. of five monoclonal autoantibodies, rheumatoid factors produced by hybridomas generated from spleen cells of BALB/c mice repeatedly infected with A/PR/8/34 human influenza A virus, revealed that they recognized distinct but spatially related epitopes. The differing isoallotypic specificity of the IgM and IgA monoclonal antibodies correlated with the presence of Ile258 and Ala305, resp. Although these data suggest that the epitopes recognized are within the CH2 domain , all antibodies failed to inhibit IIgG antigen reactivity with Staphylococcus aureus protein A (SpA), C1q, mouse C3, human FcγRI or mouse FcγRII, activities known to be predominantly determined by CH2 domain structures. Reactivity of the IgA antibody, Z34, with IgG2b allowed further specificity studies using a panel of 26 mutant IgA antibody, Z34, with IgG2b allowed further specificity studies using a panel of 26 mutant IgG2b proteins, each having single amino acid replacements over the surface of the CH2 domain. The only substitution that affected Z34 reactivity was Asn/Ala297, which destroyed the glycosylation sequon, resulting in secretion of an aglycoylated IgG mol. The epitope recognized by Z34 therefore seems to be located outside of the FcγR and C1q binding sites, but to be dependent on the presence of carbohydrate for expression. In contrast to the binding studies, complement activation by aggregated IgG2a, through classical or alternative pathways, was inhibited by the presence of autoantibodies. The functional significance of isotype-specific autoantibody in immune regulation is discussed.

L16 ANSWER 11 OF 13 CAPLUS COPYRIGHT 2008 ACS on STN

1994:161120 Document No. 120:161120 Original Reference No. 120:28364h,28365a Residue at position 331 in the IgG1 and IgG4 CH2 domains contributes to their differential ability to bind and activate complement. Xu, Yuanyuan; Oomen, Ray; Klein, Michel H. (Dep. Immunol., Univ. Toronto, Toronto, ON, M5S 1A8, Can.). Journal of Biological Chemistry, 269(5), 3469-74 (English) 1994. CODEN: JBCHA3. ISSN: 0021-9258.

AB A conserved proline residue is found at position 331 in the CH2 domains of human IgG subclasses which fix complement. This residue is replaced by a serine in IgG4 which is inactive. To determine the role of residue 331 in the differential ability of human IgGs to activate the complement cascade, a pair of genetically engineered anti-dinitrophenol IgG1 and IgG4 antibodies with reciprocal mutations at position 331 were tested for their hemolytic activity as well as for their ability to bind C1q, activate C1 and cleave C4. The IgG1 Ser331 mutant was virtually unable to mediate the lysis of trinitrobenzene-sulfonic acid-derivatized sheep red blood cells as a result of a marked defect in C1q binding activity. In contrast, the substitution of Pro for Ser331 in IgG4 bestowed partial hemolytic activity (40%) to the IgG4 Pro331 variant. Under low ionic strength conditions, this mutant was approx. 50 and 75% as active as wild-type IgG1 in the C1q binding and C4b deposition assays, resp. These results indicate that residue Pro331, which folds into close proximity to a previously identified C1q binding motif, contributes to the architecture of the IgG C1q binding site and that its replacement by a serine residue in IgG4 is largely responsible for the functional inactivity of this isotype.

L16 ANSWER 12 OF 13 CAPLUS COPYRIGHT 2008 ACS on STN

1989:207251 Document No. 110:207251 Original Reference No. 110:34286h,34287a Altered antibodies having altered effector functions and their preparation. Winter, Gregory Paul; Duncan, Alexander Robert; Burton, Dennis Raymond (Medical Research Council, UK). PCT Int. Appl. WO 8807089

A1 19880922, 42 pp. DESIGNATED STATES: W: AU, GB, JP, US; RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1988-GB211 19880318. PRIORITY: GB 1987-6425 19870318; GB 1987-18897 19870810; GB 1987-28042 19871201.

AB Antibodies (Ab) with altered binding affinity for effectors such as the Clq component of the complement system are prepared by replacing amino acid residues of the CH2 region using genetic engineering techniques. Human Cγ3 genes having a mutation, i.e. 234-leucine to alanine, 235-leuciner to glutamine, 236-glycine to alanine, and 237-glycine to alanine, resp., were constructed and cloned into expression vector pSBgpt after linking with the gene encoding the variable domain of the B18 antibody (Ab). The binding affinity I50 (the concentration of IgG3 at which the fractional binding of 125I-labeled pooled human IgG is 0.5) to Fc γ R1 receptor on U937 cells of the recombinant mutants, i.e. [234-Ala]-IgG3, [235-Glu]-IgG3, [236-Ala]-IgG3, and [237-Ala]-IgG3 were 4 + 10-8, >10-6, 3 + 10-8, and 3 + 10-7M, resp., vs. 10-8M of the control using the wild-type IgG.

L16 ANSWER 13 OF 13 CAPLUS COPYRIGHT 2008 ACS on STN

1989:73542 Document No. 110:73542 Original Reference No. 110:12123a,12126a Complement activation is not required for IgG-mediated suppression of the antibody response. Heyman, Birgitta; Wiersma, Erik; Nose, Masato (Biomed. Cent., Uppsala, Uppsala, S-75123, Swed.). European Journal of Immunology, 18(11), 1739-43 (English) 1988. CODEN: EJIMAF. ISSN: 0014-2980.

AB The authors studied whether the ability or inability of IgG antibodies to activate the complement system was of consequence for their immunosuppressive effect on the antibody response. A monoclonal IgG1-anti-2,4,6-trinitrophenyl (TNP) antibody, unable to activate complement via the classical or alternate pathway, inhibited >90% of the in vivo sheep erythrocyte-specific antibody response in mice when TNP coupled to sheep erythrocytes was used as antigen. The immunosuppressive ability was investigated of a non-complement-activating mutant IgG2a-anti-TNP monoclonal antibody. The mutant differs from the wild type by a single amino acid substitution in the CH2 domain leading to inability to fix complement factor Clq. However, the mutant has the same affinity for antigen and the same Fc receptor-binding capacity as the wild type antibody. The mutant was as efficient as the wild type antibody in inhibiting an in vitro antibody response to TNP-coupled sheep erythrocytes. These findings confirm the non-determinant specificity and Fc-dependence of IgG-mediated suppression, and show that the Fc-mediated effector mechanism is independent of complement activation. The results suggest binding to Fc receptors as a necessary step in feedback immunosuppression and favor inactivation of B cells by crosslinking of Fc and antigen receptors on their surface rather than elimination of antigen by complement-dependent phagocytosis as the effector mechanism.

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=> s l2 and deletion
L17          40 L2 AND DELETION

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L18          21 L17 AND PD<19980508

=> s l18 and RhD antigen
L19           0 L18 AND RHD ANTIGEN

=> s l17 and human platelet antigen
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L20 0 L17 AND HUMAN PLATELET ANTIGEN

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L18 ANSWER 1 OF 21 MEDLINE on STN
2000310030. PubMed ID: 10851481. A CDR-grafted (humanized) domain-deleted antitumor antibody. Slavin-Chiorini D C; Kashmiri S V; Lee H S; Milenic D E; Poole D J; Bernon E; Schlom J; Hand P H. (Laboratory of Tumor Immunology and Biology, National Cancer Institute, National Institutes of Health Bethesda, Maryland 20892, USA.) Cancer biotherapy & radiopharmaceuticals, (1997 Oct) Vol. 12, No. 5, pp. 305-16. Journal code: 9605408. ISSN: 1084-9785. Pub. country: United States. Language: English.

AB While several murine monoclonal antibodies (MAbs) directed against carcinoma associated antigens have shown excellent tumor targeting properties in clinical trials, the use of radiolabeled MAbs for both diagnostic and therapeutic applications has been hindered by two factors: (a) the induction of host anti-immunoglobulin (Ig) responses and (b) slow plasma clearance of unbound radiolabeled MAb, resulting in bone marrow toxicity for therapeutic application, and long intervals between MAb administration and tumor detection for diagnostic applications. This report describes the development of the first recombinant Ig with properties designed to reduce or eliminate both of the above problems: a complementarity determining region (CDR)-grafted humanized (Hu) MAb with a CH2 domain deletion (delta CH2). The MAb chosen for engineering was CC49, which is directed against a pancarcinoma antigen designated TAG-72 that is expressed on the majority of colorectal, gastric, breast, ovarian, prostate, pancreatic and lung carcinomas. When characterized for antigen binding in solid phase competition radioimmunoassays, the HuCC49 delta CH2 MAb completely inhibited the binding of murine (mu) CC49 and HuCC49 for TAG-72. The relative affinity constants (Ka) of MAbs HuCC49 delta CH2, HuCC49 and muCC49 were 5.1 x 10(-9), 2.1 x 10(-9) and 2.3 x 10(-9), respectively. The plasma clearance of 131I-HuCC49 delta CH2 was significantly faster than that of intact 125I-HuCC49 after either i.v. or i.p. administration in athymic mice (p(2)0.05). Biodistribution studies in athymic mice bearing human colon carcinoma xenografts after i.v. or i.p. administration of 131I-HuCC49 delta CH2 and 125I-HuCC49 demonstrated the efficient tumor localization and substantially lower percent of the injected dose (%ID/g) of the HuCC49 delta CH2 in normal tissues. This is reflected in the significantly higher radiolocalization indices (%ID/g in tumor divided by %ID/g in normal tissue) observed with the HuCC49 delta CH2 for most normal tissues tested (p(2)0.05). The differential between the rate of plasma clearance of HuCC49 delta CH2 and HuCC49 was even more pronounced in SCID mice, which have been shown to be an appropriate model to study the metabolism of human IgG. These studies thus describe the development of a recombinant Ig molecule which, for the first time, combines 1) the properties of more rapid blood clearance than an intact humanized Ig molecule--without loss of antigen binding affinity--and 2) reduced potential for eliciting a human anti-murine antibody (HAMA) response in patients. These studies also demonstrate the potential utility of HuCC49 delta CH2 for i.p. as well as i.v. radioimmunodiagnosis and radioimmunotherapy in patients with TAG-72 positive tumors.

L18 ANSWER 2 OF 21 MEDLINE on STN
96075431. PubMed ID: 7493377. Biological properties of chimeric domain-deleted anticarcinoma immunoglobulins. Slavin-Chiorini D C; Kashmiri S V; Schlom J; Calvo B; Shu L M; Schott M E; Milenic D E; Snoy P; Carrasquillo J; Anderson K; +. (Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, Maryland 20892, USA.) Cancer research, (1995 Dec 1) Vol. 55, No. 23 Suppl, pp.

5957s-5967s. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB CC49 is a second-generation monoclonal antibody (MAb) that has high affinity for the tumor-associated pancarcinoma antigen tumor-associated glycoprotein-72. In clinical trials using gamma scanning, radiolabeled CC49 has facilitated the detection of more than 90% of carcinomas. We report here the development of a constant heavy-chain 2 (CH2) domain-deleted chimeric (c) CC49 MAb by transfecting an expression construct consisting of the CC49 murine variable region and a CH2 domain-deleted human IgG1 constant region into cCC49 kappa producing SP2/0 murine myeloma cells. As determined by SDS-PAGE, the intact cCC49 delta CH2 has a molecular weight of 153,000 and, under reducing conditions, molecular weights of 43,000 and 27,000. The plasma clearance and tumor-targeting properties of cCC49 delta CH2 were evaluated and compared with those of mouse/human chimeric forms cCC49 delta CH1 and intact cCC49. Previous studies have shown that the in vitro antigen-binding properties of cCC49 delta CH1 are similar to those of cCC49. Biodistribution studies reported here, using 131I-labeled cCC49 delta CH1 and 125I-labeled cCC49 in athymic mice bearing human colon carcinoma xenografts, demonstrated that both cMAbs localized to the tumor and cleared from the normal tissues similarly. However, in comparison with 125I-labeled cCC49, 131I-labeled cCC49 delta CH2 localized to tumors earlier and had a significantly lower percentage of the injected dose of cMAb/g (%ID/g) in normal tissues than cCC49. Immunoscintigraphy of 131I-labeled cCC49 delta CH2 and 125I-labeled cCC49 in athymic mice bearing human tumor xenografts demonstrated a clear image of the tumor by 24 h after i.v. administration of the delta CH2 cMAb versus the 72 h required for cCC49. Biodistribution studies using 177Lu-conjugated cCC49 delta CH1 and cCC49 showed no significant difference between the radiolocalization indices (% ID/g in tumor divided by % ID/g in normal tissue). 177Lu-conjugated cCC49 delta CH2, however, had lower % ID/g values in tumor xenografts and lower radiolocalization indices than either 177Lu-conjugated cCC49 delta CH1 or 177Lu-conjugated cCC49. Pharmacokinetic studies in non-tumor-bearing athymic mice using cCC49 delta CH1 and cCC49 revealed no significant difference between these cMAbs. However, the plasma clearance of cCC49 delta CH2 in non-tumor-bearing mice was significantly faster than that of cCC49. These results were similar when the cMAbs were labeled with either iodine or lutetium. In nonhuman primates, 131I-labeled cCC49 delta CH2 cleared significantly faster than 125I-labeled cCC49. The similar plasma clearance and tumor localization of cCC49 and cCC49 delta CH1 suggest that these two cMAbs may be used in similar clinical settings. However, because of the unique pharmacokinetics and tumor targeting of cCC49 delta CH2 versus cCC49 or cCC49 delta CH1, this chimeric immunoglobulin form may be useful in clinical settings that require efficient tumor targeting and rapid serum and whole-body clearance.

L18 ANSWER 3 OF 21 MEDLINE on STN

94043382. PubMed ID: 8227075. Identification of a secondary Fc gamma RI binding site within a genetically engineered human IgG antibody. Chappel M S; Isenman D E; Oomen R; Xu Y Y; Klein M H. (Department of Immunology, University of Toronto, Ontario, Canada.) The Journal of biological chemistry, (1993 Nov 25) Vol. 268, No. 33, pp. 25124-31. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Although human IgG2 is not cytophilic, we have shown previously that an IgG2 antibody expressing the sequence PLLGG (underline = substitution) spanning CH2 domain residues 233-237 (Eu numbering) displayed IgG1-like Fc gamma RI binding activity. In contrast, IgG1 PLLGG exhibited 3-fold less affinity, whereas IgG2 ELLGG was 3-fold more active than native IgG1. These results suggested that additional

site(s) conferred enhanced binding properties to the engineered, cytophilic IgG2 variant. These sites were shown to reside in the IgG2 CH2 domain, since the IgG1 CH2 module did not have enhanced activity in a panel of hybrid IgG1/IgG2 antibodies. To map these sites further, human IgG1 and IgG2 constant region gene segments were modified to allow reciprocal COOH-terminal half segment exchanges of CH2 exons. These were cloned into a pSV2neo expression vector bearing a rearranged MOPC 315 heavy chain variable region gene and transfected into a MOPC 315 heavy chain deletion mutant. The dinitrophenol affinity-purified IgGs were radiolabeled and assessed for Fc gamma RI binding activity in direct binding assays using U937 cells. The COOH terminus of the IgG2 CH2 domain was found to contain accessory site(s) since it enhanced the binding properties of both IgG1 PLLGG and native IgG1. In contrast, grafting of the COOH terminus of the IgG1 CH2 domain onto IgG2 PLLGG and IgG2 ELLGG diminished their cytophilic activity. The amino acid responsible for the enhancing properties of the COOH terminus of the IgG2 CH2 domain was shown to be threonine 339, since IgG1 PLLGG/Thr339 displayed increased Fc gamma RI binding affinity. Kinetics studies revealed that this is accomplished through an increase in the forward rate constant of the IgG-Fc gamma RI interaction.

L18 ANSWER 4 OF 21 MEDLINE on STN

93155484. PubMed ID: 7679128. Epitope mapping of human immunoglobulin-specific murine monoclonal antibodies with domain-switched, deleted and point-mutated chimeric antibodies.

Hamilton R G; Morrison S L. (Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21224.) Journal of immunological methods, (1993 Jan 14) Vol. 158, No. 1, pp. 107-22. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB 27 engineered chimeric antibodies possessing human gamma, epsilon, mu or alpha constant regions and V region specificity for nitrophenyl or dansyl were used to study the isotype specificity of 29 murine monoclonal antibodies (MAbs) specific for human immunoglobulins (IgG1-4, IgE, IgM, IgA or secretory piece). The isotype-restricted immunoreactivity observed with wild-type chimeric antibodies paralleled the pattern of each MAb's reactivity with purified human myeloma proteins. 16 mutant IgG anti-dansyl chimeric antibodies with genetically engineered domain switches, deletions or point-mutations were used as antigens to further characterize the epitopes recognized by the human IgG subclass-specific MAbs. The binding of three human IgG1-specific MAbs (HP6069, HP6070 and HP6091) was mapped to similar epitopes on the CH2 domain of human IgG1. Of the two anti-human IgG2 MAbs tested, HP6002 reacted with the CH2 of IgG2 while HP6014 bound to the CH1 domain. Both anti-human IgG3 MAbs (HP6047, HP6050) reacted with different regions of the IgG3 hinge. The anti-human IgG4 MAbs (HP6023, HP6025) bound to a similar epitope on the carboxyl terminus of CH2 or the CH3 of human IgG4. The three exclusion antibodies (HP6019, HP6030 and HP6058) bound to different epitopes in the CH2 domain of three of four IgG subclasses. The domain mapping was confirmed by competitive inhibition experiments. These results were used to select a group of IgG-reactive MAbs for construction of a poly-monoclonal anti-IgG capture and detection reagent that uniformly bound all four subclasses of human IgG. This study provides support for the use of engineered chimeric human chimeric antibodies as replacements for increasingly rare, purified human paraproteins in the specificity analysis of immunochemical reagents used in clinical and research laboratories for the detection and quantitation of human antibodies. Moreover, these studies demonstrate how the MAbs can serve as effective probes for examining

conformational differences among the four human IgG subclasses.

L18 ANSWER 5 OF 21 MEDLINE on STN
93093838. PubMed ID: 8416208. Biologic properties of a CH2 domain-deleted recombinant immunoglobulin. Slavin-Chiorini D C; Horan Hand P H; Kashmiri S V; Calvo B; Zaremba S; Schlom J. (Laboratory of Tumor Immunology and Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.) International journal of cancer. Journal international du cancer, (1993 Jan 2) Vol. 53, No. 1, pp. 97-103. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.
AB Monoclonal antibody (MAb) B72.3 reacts with TAG-72, a high-molecular-weight mucin expressed on several types of human carcinoma, and is currently being used in clinical trials for the diagnosis and therapy of human carcinoma. An expression construct containing cDNA encoding an immunoglobulin (Ig) heavy chain, with the variable region of murine MAb B72.3 and a human Ig constant region with a deletion of the CH2 domain, was generated. Immunoglobulin from the transfectoma with the highest expression of the TAG-72 immunoreactive antibody was designated MAb chimeric (c) B72.3 delta CH2. The pharmacokinetics of serum clearance of iodine-labeled MAbs cB72.3 delta CH2 and the intact cB72.3 were compared in athymic mice. By 24 hr, less than 1% of the cB72.3 delta CH2 was left in the plasma, while 36% of the cB72.3 still remained. The T1/2 alpha values of the cB72.3 delta CH2 and cB72.3 MAbs were 1.7 and 2.4 hr, respectively. The T1/2 beta values were 7.8 hr for the domain-deleted cMAb and 48.9 hr for cB72.3. Biodistribution studies in athymic mice bearing LS-174T xenografts showed a reduction in the percentage of injected dose per gram in tumor with 131I-cB72.3 delta CH2; however, the 131I-cB72.3 delta CH2 both localized to tumors faster and cleared from the blood faster than the 125I-cB72.3 MAb. Only trace amounts of the 131I-cB72.3 delta CH2 were detected in normal tissues, including kidney. The faster clearance rate, more rapid tumor targeting and lack of metabolic uptake in normal tissues demonstrated with the iodine-labeled CH2 domain -deleted cMAb may be an advantage for certain clinical protocols.

L18 ANSWER 6 OF 21 MEDLINE on STN
91324713. PubMed ID: 1713936. Identification of epitopes recognized by a panel of six anti-human IgG2 monoclonal antibodies. Harada S; Hata S; Kosada Y; Kondo E. (Shionogi Institute for Medical Science, Osaka, Japan.) Journal of immunological methods, (1991 Jul 26) Vol. 141, No. 1, pp. 89-96. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.
AB Human IgG2 contains several subclass specific amino acid residues or deletions in the CH1 and CH2 domains and also in the hinge region. These substituted residues are the structural correlates for IgG2 specific epitopes. Since human IgG2 has different biological properties from other subclasses, some IgG2 epitopes may be located in regions correlating with sites determining the biological functions. Previously, we produced three anti-IgG2 monoclonal antibodies (mAbs) with highly specific and interesting reactivities using improved immunization protocols. However, it has been almost impossible to identify epitopes conventionally, because human IgG2 is so resistant to proteolysis that various proteolytic fragments could not be isolated. In this study, we identified the epitopes recognized by anti-IgG2 mAbs by SDS-PAGE, Western blotting, amino acid sequence analysis and peptide/mAb binding ELISA, thus overcoming the need for fragment isolation. A panel of six anti-human IgG2 mAbs, including the current WHO/IUIS specificity standards (HP6002, HP6008, HP6014) and our own (HG2-6A, HG2-30F, HG2-56F), reacted with distinct epitopes. The residues essential to expression of the epitopes recognized by the mAbs were:

Pro234, Val235 and Val309 for HG2-56F, HG2-30F and HP6008, respectively. HP6014 reacted with the epitope expressed by Thr214 and its neighboring residues. HG2-6A was reactive with the hinge region, and HP6002 was assumed to be directed against discontinuous epitopes requiring intact Fc for expression. Through these studies, the pepsin and papain cleavage sites of human IgG2 were also clarified.

L18 ANSWER 7 OF 21 MEDLINE on STN
90332649. PubMed ID: 2198570. Serum half-life and tumor localization of a chimeric antibody deleted of the CH2 domain and directed against the disialoganglioside GD2. Mueller B M; Reisfeld R A; Gillies S D. (Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.) Proceedings of the National Academy of Sciences of the United States of America, (1990 Aug) Vol. 87, No. 15, pp. 5702-5. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB Recombinant techniques allow one to engineer an antibody molecule and, in this way, manipulate its properties and functions. We engineered a chimeric human/mouse antibody to the tumor-associated antigen ganglioside GD2, with the aim of decreasing its serum half-life, maintaining its full antigen-binding capacity, and deleting its effector functions, thus making it a potentially useful reagent for the radioimaging of tumors. To this end, the constant region of the human gamma 1 chain was mutated by deleting the second domain (CH2). Here we show that the CH2-deleted antibody (ch14.18-delta CH2) was cleared from the blood of athymic (nu/nu) mice bearing human melanoma tumors with the same kinetics as human IgG F(ab')2. At a beta t1/2 of 12 hr, 0.9% of the injected dose of 125I-labeled ch14.18-delta CH2 was found per milliliter of blood 24 hr after i.v. injection. In biodistribution experiments, 125I-labeled ch14.18-delta CH2 targeted specifically to melanoma xenografts, achieving optimal tumor-to-tissue ratios 12-16 hr after i.v. injection. ch14.18-delta CH2 was localized to the melanoma tumors more rapidly and with better localization ratios than the intact chimeric antibody ch14.18. Sixteen hours after i.v. injection, the tumor-to-blood and tumor-to-liver ratios of ch14.18-delta CH2 were 5 and 12, respectively, while optimal localization ratios obtained for ch14.18 were 1 and 5, respectively, but 96 hr after injection. A reagent such as ch14.18-delta CH2 should be useful for radioimmunodetection of human tumors because of reduced immunogenicity, increased targeting specificity, and rapid clearance from circulation.

L18 ANSWER 8 OF 21 MEDLINE on STN
84034926. PubMed ID: 6195294. Determinants recognized by murine rheumatoid factors: molecular localization using a panel of mouse myeloma variant immunoglobulins. Stassin V; Coulie P G; Birshtein B K; Secher D S; Van Snick J. The Journal of experimental medicine, (1983 Nov 1) Vol. 158, No. 5, pp. 1763-8. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.
AB The structures recognized by monoclonal anti-IgG1 rheumatoid factors (RF) were localized by testing their reactivity with mutant immunoglobulins carrying gamma 1 chains that lacked either the CH1 or the CH3 domains. While optimal binding was observed in the absence of CH1, deletion of CH3 completely abolished the reactivity of all but one of the 71 monoclonal RF tested. Similar experiments were carried out with IgG2a- and IgG2b-specific RF by using variant immunoglobulins carrying various hybrid gamma 2a-gamma 2b heavy chains. It was found that both the polyclonal RF produced by autoimmune strains, MRL/MpJ-lpr and NZB/BinJ, and most of the monoclonal RF derived from normal strains, BALB/c, C57Bl/6, and 129/Sv, were directed against determinants located in a segment spanning the C-terminal 8 residues of the CH2

domain and the complete CH3 domain.

L18 ANSWER 9 OF 21 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN
1998004618 EMBASE A CDR-grafted (humanized) domain-deleted antitumor antibody.
Slavin-Chiorini, Dale C.; Kashmiri, Syed V.S.; Milenic, Diane E.; Poole, Diane J.; Bernon, Eric; Schlom, Jeffrey; Horan Hand, Patricia. Lab. of Tumor Immunology and Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD, United States. Lee, Hyun-Sil. Protein Engineering Research Group, Korean Res. Inst. Biosci. Biotech., Taejon 305-600, Korea, Republic of. Horan Hand, Patricia. Lab. of Tumor Immunology and Biology, National Cancer Institute, NIH, Bethesda, MD 20892, United States. Hand, P.H. (correspondence). Lab. of Tumor Immunology and Biology, National Cancer Institute, Building 10, Bethesda, MD 20892, United States.
Cancer Biotherapy and Radiopharmaceuticals Vol. 12, No. 5, pp. 305-316 Oct 1997.
Refs: 32.
ISSN: 1084-9785. CODEN: CBRAFJ
Pub. Country: United States. Language: English. Summary Language: English.
Entered STN: 19980122. Last Updated on STN: 19980122
AB While several murine monoclonal antibodies (MAbs) directed against carcinoma associated antigens have shown excellent tumor targeting properties in clinical trials, the use of radiolabeled MAbs for both diagnostic and therapeutic applications has been hindered by two factors: (a) the induction of host anti-immunoglobulin (Ig) responses and (b) slow plasma clearance of unbound radiolabeled MAb, resulting in bone marrow toxicity for therapeutic application, and long intervals between MAb administration and tumor detection for diagnostic applications. This report describes the development of the first recombinant Ig with properties designed to reduce or eliminate both of the above problems: a complementarity determining region (CDR)- grafted humanized (Hu) MAb with a CH2 domain deletion (ΔCH2). The MAb
chosen for engineering was CC49, which is directed against apancarcinoma antigen designated TAG-72 that is expressed on the majority of colorectal, gastric, breast, ovarian, prostate, pancreatic and lung carcinomas. When characterized for antigen binding in solid phase competition radioimmunoassays, the HuCC49 ΔCH2 MAb completely inhibited the binding of murine (mu) CC49 and HuCC49 for TAG-72. The relative affinity constants (K(a)) of MAbs HuCC49ΔCCHuCC49 and muCC49 were 5.1 x 10(-9), 2.1 x 10(-9) and 2. 3 x 10(-9), respectively. The plasma clearance of I-HuCC49 ΔCH2 was significantly faster than that of intact (125)-HuCC49 after either i v. or i.p. administration in athymic mice (p(2)O. 05). Biodistribution studies in athymic mice bearing human colon carcinoma xenografts after i.v. ori.p. administration of (131)-HuCC49ΔCH2 and (125)I-HuCC49 demonstrated the efficient tumor localization and substantially lower percent of the injected dose (%ID/g) of the HuCC49ΔCH2 in normal tissues. This is reflected in the significantly higher radiolocalization indices (%ID/g in tumor divided by %ID/g in normal tissue) observed with the HuCC49ΔCH2 for most normal tissues tested (p(2)0.05). The differential between the rate of plasma clearance of HuCC49 ΔCH2 and HuCC49 was even more pronounced in SCID mice, which have been shown to be an appropriate model to study the metabolism of human IgG. These studies thus describe the development of a recombinant Ig molecule which, for the first time, combines 1) the properties of more rapid blood clearance than an intact humanized Ig molecule without loss of antigen binding affinity and 2) reduced potential for eliciting a human anti-imurine antibody (HAMA) response in patients. These studies also demonstrate the potential utility of HuCC49 ΔCH2 for i.p. as well as i.v. radioimmunodiagnosis and

radioimmunotherapy in patients with TAG-72 positive tumors.

L18 ANSWER 10 OF 21 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
1993:186789 Document No.: PREV199395097239. Epitope mapping of human immunoglobulin-specific murine monoclonal antibodies with domain-switched, deleted and point-mutated chimeric antibodies. Hamilton, Robert G. [Reprint author]; Morrison, Sherie L.. Room 1A20, DACI Laboratory, Johns Hopkins Asthma Allergy Center, 5501 Hopkins Bayview Circle, Baltimore, MD 21224, USA. Journal of Immunological Methods, (1993) Vol. 158, No. 1, pp. 107-122.
CODEN: JIMMBG. ISSN: 0022-1759. Language: English.
AB 27 engineered chimeric antibodies possessing human gamma, epsilon, mu or alpha constant regions and V region specificity for nitrophenyl or dansyl were used to study the isotype specificity of 29 murine monoclonal antibodies (MAbs) specific for human immunoglobulins (IgG1-4, IgE, IgM, IgA or secretory piece). The isotype-restricted immunoreactivity observed with wild-type chimeric antibodies paralleled the pattern of each MAb's reactivity with purified human myeloma proteins. 16 mutant IgG anti-dansyl chimeric antibodies with genetically engineered domain switches, deletions or point-mutations were used as antigens to further characterize the epitopes recognized by the human IgG subclass-specific MAbs. The binding of three human IgG1-specific MAbs (HP6069, HP6070 and HP6091) was mapped to similar epitopes on the C-H2 domain of human IgG1. Of the two anti-human IgG2 MAbs tested, HP6002 reacted with the C-H2 of IgG2 while HP6014 bound to the C-H1 domain. Both anti-human IgG3 MAbs (HP6047, HP6050) reacted with different region of the IgG3 hinge. The anti-human IgG4 MAbs (HP6023, HP6025) bound to a similar epitope on the carboxyl terminus of C-H2 or the C-H3 of human IgG4. The three exclusion antibodies (HP6019, HP6030 and HP6058) bound to different epitopes in the C-H2 domain of three of four IgG subclasses. The domain mapping was confirmed by competitive inhibition experiments. These results were used to select a group of IgG-reactive MAbs for construction of a poly-monoclonal anti-IgG capture and detection reagent that uniformly bound all four subclasses of human IgG. This study provides support for the use of engineered chimeric human chimeric antibodies as replacements for increasingly rare, purified human paraproteins in the specificity analysis of immunochemical reagents used in clinical and research laboratories for the detection and quantitation of human antibodies. Moreover, these studies demonstrate how the MAbs can serve as effective probes for examining conformational differences among the four human IgG subclasses.

L18 ANSWER 11 OF 21 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
1992:362142 Document No.: PREV199243040292; BR43:40292. RADIOIMMUNOTHERAPY POTENTIAL OF CH2 DOMAIN DELETION MUTANT OF NU-LU-10 COMPARED TO MAB AND FAB'2. BEAUMIER P L [Reprint author]; BOTTINO B; MCINTYRE R; LIBBY D; RENO J M. NEORX CORP, SEATTLE, WASH, USA. Journal of Nuclear Medicine, (1992) Vol. 33, No. 5 SUPPL, pp. 1028.
Meeting Info.: 39TH ANNUAL MEETING OF THE SOCIETY OF NUCLEAR MEDICINE, LOS ANGELES, CALIFORNIA, USA, JUNE 9-12, 1992. J NUCL MED.
CODEN: JNMEAQ. ISSN: 0161-5505. Language: ENGLISH.

L18 ANSWER 12 OF 21 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
1991:455313 Document No.: PREV199192100093; BA92:100093. IDENTIFICATION OF EPITOPES RECOGNIZED BY A PANEL OF SIX ANTI-HUMAN IGG2 MONOCLONAL ANTIBODIES. HARADA S [Reprint author]; HATA S; KOSADA Y; KONDO E. SHIONOGI INST MED SCI, 2-5-1 MISHIMA, SETTSU-SHI, OSAKA 566, JPN. Journal

of Immunological Methods, (1991) Vol. 141, No. 1, pp. 89-96.
CODEN: JIMMBG. ISSN: 0022-1759. Language: ENGLISH.

AB Human IgG2 contains several subclass specific amino acid residues or deletions in the CH1 and CH2 domains and also in the hinge region. These substituted residues are the structural correlates for IgG2 specific epitopes. Since human IgG2 has different biological properties from other subclasses, some IgG2 epitopes may be located in regions correlating with sites determining the biological functions. Previously, we produced three anti-IgG2 monoclonal antibodies (mAbs) with highly specific and interesting reactivities using improved immunization protocols. However, it has been almost impossible to identify epitopes conventionally, because human IgG2 is so resistant to proteolysis that various proteolytic fragments could not be isolated. In this study, we identified the epitopes recognized by anti-IgG2 mAbs by SDS-PAGE, Western blotting, amino acid sequence analysis and peptide/mAb binding ELISA, thus overcoming the need for fragment isolation. A panel of six anti-human IgG2 mAbs, including the current WHO/IUIS specificity standards (HP6002, HP6008, HP6014) and our own (HG2-6A, HG2-30F, HG2-56F), reacted with distinct epitopes. The residues essential to expression of the epitopes recognized by the mAbs were: Pro234, Val235 and Val309 for HG2-56F, HG2-30F and HP6008, respectively. HP6014 reacted with the epitope expressed by Thr214 and its neighboring residues. HG2-6A was reactive with the hinge region, and HP6002 was assumed to be directed against discontinuous epitopes requiring intact Fc for expression. Through these studies, the pepsin and papain cleavage sites of human IgG2 were also clarified.

L18 ANSWER 13 OF 21 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

1997:920513 The Genuine Article (R) Number: YL430. A CDR-grafted (humanized) domain-deleted antitumor antibody. SlavinChiorini D C (Reprint); Kashmiri S V S; Lee H S; Milenic D E; Poole D J; Bernon E; Schlom J; Hand P H. NCI, TUMOR IMMUNOL & BIOL LAB, NIH, BETHESDA, MD 20892. CANCER BIOTHERAPY AND RADIOPHARMACEUTICALS (OCT 1997) Vol. 12, No. 5, pp. 305-316. ISSN: 1084-9785. Publisher: MARY ANN LIEBERT INC PUBL, 2 MADISON AVENUE, LARCHMONT, NY 10538. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB While several murine monoclonal antibodies (MAbs) directed against carcinoma associated antigens have shown excellent tumor targeting properties in clinical trials, the use of radiolabelled MAbs for both diagnostic and therapeutic applications has been hindered by two factors: (a) the induction of host anti-immunoglobulin (Ig) responses and (b) slow plasma clearance of unbound radiolabelled MAb, resulting in bone marrow toxicity for therapeutic application, and long intervals between MAb administration and tumor detection for diagnostic applications. This report describes the development of the first recombinant Ig with properties designed to reduce or eliminate both of the above problems: a complementarity determining region (CDR)-grafted humanized (Hu) MAb with a CH2 domain deletion (Delta CH2). The MAb chosen for engineering was CC49, which is directed against a pancarcinoma antigen designated TAG-72 that is expressed on the majority of colorectal, gastric, breast ovarian, prostate, pancreatic and lung carcinomas. When characterized for antigen binding in solid phase competition radioimmunoassays, the HuCC49 Delta CH2 MAb completely inhibited the binding of murine (mu) CC49 and HuCC49 for TAG-72. The relative affinity constants (K-a) of MAbs HuCC49 Delta CH2, HuCC49 and muCC49 were 5.1 x 10(-9), 2.1 x 10(-9) and 2.3 x 10(-9) respectively. The plasma clearance of I-131-HuCC49 Delta CH2 was significantly faster than that of intact I-125-HuCC49 after either i.v. or i.p. administration in athymic mice (p(2)0.05). Biodistribution studies in athymic mice bearing human colon carcinoma xenografts after i.v. or ip. administration of I-131-HuCC49

Delta CH2 and I-125-HuCC49 demonstrated the efficient tumor localization and substantially lower percent of the injected dose (%ID/g) of the HuCC49 Delta CH2 in normal tissues. This is reflected in the significantly higher radiolocalization indices (%ID/g in tumor divided by %ID/g in normal tissue) observed with the HuCC49 Delta CH2 for most normal tissues tested (p(2)0.05). The differential between the rate of plasma clearance of HuCC49 Delta CH2 and HuCC49 was even more pronounced in SCID mice, which have been shown to be an appropriate model to study the metabolism of human IgG. These studies thus describe the development of a recombinant Ig molecule which, for the first time, combines 1) the properties of more rapid blood clearance than an intact humanized Ig molecule-without loss of antigen binding affinity-and 2) reduced potential for eliciting a human anti-murine antibody (HAMA) response in patients. These studies also demonstrate the potential utility of HuCC49 Delta CH2 for i.p. as well as i.v. radioimmunodiagnosis and radioimmunotherapy in patients with TAG-72 positive tumors.

L18 ANSWER 14 OF 21 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

1993:13156 The Genuine Article (R) Number: KD949. BIOLOGIC PROPERTIES OF A CH2 DOMAIN-DELETED RECOMBINANT IMMUNOGLOBULIN.
SLAVINCHIORINI D C (Reprint); HAND P H; KASHMIRI S V S; CALVO B; ZAREMBA S; SCHLOM J. NCI, TUMOR IMMUNOL & BIOL LAB, BLDG 10, ROOM 8B07, 9000 ROCKVILLE PIKE, BETHESDA, MD 20892. INTERNATIONAL JOURNAL OF CANCER (2 JAN 1993) Vol. 53, No. 1, pp. 97-103. ISSN: 0020-7136.
Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC, 111 RIVER ST, HOBOKEN, NJ 07030 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Monoclonal antibody (MAb) B72.3 reacts with TAG-72, a high-molecular-weight mucin expressed on several types of human carcinoma, and is currently being used in clinical trials for the diagnosis and therapy of human carcinoma. An expression construct containing cDNA encoding an immunoglobulin (Ig) heavy chain, with the variable region of murine MAb B72.3 and a human Ig constant region with a deletion of the C(H)2 domain, was generated. Immunoglobulin from the transfectoma with the highest expression of the TAG-72 immunoreactive antibody was designated MAb chimeric (c) B72.3DELTAC(H)2. The pharmacokinetics of serum clearance of iodine-labeled MAbs cB72.3DELTAC(H)2 and the intact cB72.3 were compared in athymic mice. By 24 hr, less than 1 % of the cB72.3DELTAC(H)2 was left in the plasma, while 36% of the cB72.3 still remained. The T1/2alpha values of the cB72.3DELTAC(H)2 and cB72.3 MAbs were 1.7 and 2.4 hr, respectively. The T1/2beta values were 7.8 hr for the domain-deleted cMAb and 48.9 hr for cB72.3. Biodistribution studies in athymic mice bearing LS-174T xenografts showed a reduction in the percentage of injected dose per gram in tumor with I-131-cB72.3DELTAC(H)2; however, the I-131-cB72.3DELTAC(H)2 both localized to tumors faster and cleared from the blood faster than the I-125-cB72.3 MAb. Only trace amounts of the I-131-cB72.3DELTAC(H)2 were detected in normal tissues, including kidney. The faster clearance rate, more rapid tumor targeting and lack of metabolic uptake in normal tissues demonstrated with the iodine-labeled C(H)2 domain-deleted cMAb may be an advantage for certain clinical protocols.

L18 ANSWER 15 OF 21 CAPLUS COPYRIGHT 2008 ACS on STN

1998:23700 Document No. 128:87645 A CDR-grafted (humanized) domain-deleted antitumor antibody. Slavin-Chiorini, Dale C.; Kashmiri, Syed V. S.; Lee, Hyun-Sil; Milenic, Diane E.; Poole, Diane J.; Bernon, Eric; Schlom, Jeffrey; Hand, Patricia Horan (Laboratory of Tumor Immunology and Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892, USA). Cancer Biotherapy & Radiopharmaceuticals, 12(5), 305-316 (English) 1997. CODEN: CBRAFJ. ISSN: 1084-9785.

Publisher: Mary Ann Liebert, Inc..

AB While several murine monoclonal antibodies (MAbs) directed against carcinoma associated antigens have shown excellent tumor targeting properties in clin. trials, the use of radiolabeled MAbs for both diagnostic and therapeutic applications has been hindered by two factors: (a) the induction of host anti-Ig responses and (b) slow plasma clearance of unbound radiolabeled MAb, resulting in bone marrow toxicity for therapeutic application, and long intervals between MAb administration and tumor detection for diagnostic applications. This report describes the development of the first recombinant Ig with properties designed to reduce or eliminate both of the above problems: a complementarity determining region (CDR)-grafted humanized (Hu) MAb with a CH2 domain deletion (ΔCH2). The MAb chosen for engineering was CC49, which is directed against a pancarcinoma antigen designated TAG-72 that is expressed on the majority of colorectal, gastric, breast, ovarian, prostate, pancreatic and lung carcinomas. When characterized for antigen binding in solid phase competition RIAs, the HuCC49ΔCH2 MAb completely inhibited the binding of murine (mu) CC49 and HuCC49 for TAG-72. The relative affinity consts. (Ka) of MAbs HuCC49ΔCH2, HuCC49 and muCC49 were 5.1+10-9, 2.1+10-9 and 2.3+10-9, resp. The plasma clearance of 131 I-HuCC49ΔCH2 was significantly faster than that of intact 125I-HuCC49 after either i.v. or i.p. administration in athymic mice (p20.05). Biodistribution studies in athymic mice bearing human colon carcinoma xenografts after i.v. or i.p. administration of 131I-HuCC49ΔCH2 and 125I-HuCC49 demonstrated the efficient tumor localization and substantially lower percent of the injected dose (%ID/g) of the HuCC49ΔCH2 in normal tissues. This is reflected in the significantly higher radiolocalization indexes (%ID/g in tumor divided by %ID/g in normal tissue) observed with the HuCC49ΔCH2 for most normal tissues tested (p20.05). The differential between the rate of plasma clearance of HuCC49ΔCH2 and HuCC49 was even more pronounced in SCID mice, which have been shown to be an appropriate model to study the metabolism of human IgG. These studies thus describe the development of a recombinant Ig mol. which, for the first time, combines (1) the properties of more rapid blood clearance than an intact humanized Ig mol. - without loss of antigen binding affinity - and (2) reduced potential for eliciting a human anti-murine antibody (HAMA) response in patients. These studies also demonstrate the potential utility of HuCC49ΔCH2 for i.p. as well as i.v. radioimmunodiagnosis and radioimmunotherapy in patients with TAG-72 pos. tumors.

L18 ANSWER 16 OF 21 CAPLUS COPYRIGHT 2008 ACS on STN

1993:623788 Document No. 119:223788 Original Reference No. 119:39901a,39904a Identification of a secondary FcγRI binding site within a genetically engineered human IgG antibody. Chappel, M. Suzanne; Isenman, David E.; Oomen, R.; Xu, Yuan Yuan; Klein, Michel H. (Dep. Immunol., Univ. Toronto, Toronto, ON, M5S 1A8, Can.). Journal of Biological Chemistry, 268(33), 25124-31 (English) 1993. CODEN: JBCHA3. ISSN: 0021-9258.

AB Although human IgG2 is not cytophilic, the authors have shown previously that an IgG2 antibody expressing the sequence PLLGG spanning CH2 domain residues 233-237 (Eu numbering) displayed IgG1-like FcγRI binding activity. In contrast, IgG1 PLLGG exhibited 3-fold less affinity, whereas IgG2 ELLGG was 3-fold more active than native IgG1. These results suggested that addnl. site(s) conferred enhanced binding properties to the engineered, cytophilic IgG2 variant. These sites were shown to reside in the IgG2 CH2 domain , since the IgG1 CH2 module did not have enhanced activity in a panel of hybrid IgG1/IgG2 antibodies. To map these sites further human IgG1 and IgG2 constant region gene segments were modified to allow reciprocal C-terminal half segment exchanges of CH2 exons. These were

cloned into a pSV2neo expression vector bearing a rearranged MOPC 315 heavy chain variable region gene and transfected into a MOPC 315 heavy chain deletion mutant. The dinitrophenyl affinity-purified IgGs were radiolabeled and assessed for FcγRI binding activity in direct binding assays using U937 cells. The C-terminus of the IgG2 CH2 domain was found to contain accessory site(s) since it enhanced the binding properties of both IgG1 PLLGG and native IgG1. In contrast, grafting of the C-terminus of the IgG1 CH2 domain onto IgG2 PLLGG and IgG2 ELLGG diminished their cytophilic activity. The amino acid responsible for the enhancing properties of the C-terminus of the IgG2 CH2 domain was shown to be threonine 339, since IgG1 PLLGG/Thr339 displayed increased FcγRI binding affinity. Kinetic studies revealed that this is accomplished through an increase in the forward rate constant of the IgG-FcγRI interaction.

L18 ANSWER 17 OF 21 CAPLUS COPYRIGHT 2008 ACS on STN
1993:187047 Document No. 118:187047 Original Reference No. 118:32015a,32018a
Biological properties of a CH2 domain-deleted recombinant immunoglobulin. Slavin-Chiorini, Dale C.; Hand, Patricia Horan; Kashmiri, S. V. S.; Calvo, Benjamin; Zaremba, Sam; Schlom, Jeffrey (Lab. Tumor Immunol. Biol., Natl. Cancer Inst., Bethesda, MD, 20892, USA). International Journal of Cancer, 53(1), 97-103 (English) 1993. CODEN: IJCNAW. ISSN: 0020-7136.

AB Monoclonal antibody (MAb) B72.3 reacts with TAG-72, a high-mol.-weight mucin expressed on several types of human carcinoma, and is currently being used in clin. trials for the diagnosis and therapy of human carcinoma. An expression construct containing cDNA encoding an Ig heavy chain, with the variable region of murine MAb B72.3 and a human Ig constant region with a deletion of the CH2 domain, was generated. Ig from the transfectoma with the highest expression of the TAG-72 immunoreactive antibody was designated MAb chimeric (c) B72.3ΔCH2. The pharmacokinetics of serum clearance of iodine-labeled MAbs cB72.3ΔCH2 and the intact cB72.3 were compared in athymic mice. By 24 h, <1% of the cB72.3ΔCH2 was left in the plasma, while 36% of the cB72.3 still remained. The T1/2α values of the cB72.3ΔCH2 and cB72.3 MAbs were 1.7 and 2.4 h, resp. The T1/2β values were 7.8 h for the domain-deleted cMAb and 48.9 h for cB72.3. Biodistribution studies in athymic mice bearing LS-174T xenografts showed a reduction in the percent of injected dose per g in tumor with 131I-cB72.3ΔCH2; however, the 131I-cB72.3ΔCH2 both localized to tumors faster and cleared from the blood faster than the 125I-cB72.3 MAb. Only trace amts. of the 131I-cB72.3ΔCH2 were detected in normal tissues, including kidney. The faster clearance rate, more rapid tumor targeting and lack of metabolic uptake in normal tissues demonstrated with the iodine-labeled CH2 domain -deleted cMAb may be an advantage for certain clin. protocols.

L18 ANSWER 18 OF 21 CAPLUS COPYRIGHT 2008 ACS on STN
1993:166980 Document No. 118:166980 Original Reference No. 118:28601a,28604a
Epitope mapping of human immunoglobulin-specific murine monoclonal antibodies with domain-switched, deleted and point-mutated chimeric antibodies. Hamilton, Robert G.; Morrison, Sherie L. (Div. Clin. Immunol., Dep. Med., Johns Hopkins Univ. Sch. Med., Baltimore, MD, 21224, USA). Journal of Immunological Methods, 158(1), 107-22 (English) 1993. CODEN: JIMMBG. ISSN: 0022-1759.

AB Some 27 engineered chimeric antibodies possessing human γ, ε, μ, or α constant regions and V region specificity for nitrophenyl or dansyl were used to study the isotype specificity of 29 murine monoclonal antibodies (MAbs) specific for human Igs (IgG1-4, IgE, IgM, IgA, or secretory piece). The isotype-restricted immunoreactivity observed with wild-type chimeric antibodies

paralleled the pattern of each MAb's reactivity with purified human myeloma proteins. Sixteen mutant IgG anti-dansyl chimeric antibodies with genetically engineered domain switches, deletions, or point mutations were used as antigens to further characterize the epitopes recognized by the human IgG subclass-specific MAbs. The binding of three human IgG1-specific MAbs (HP6069, HP6070, and HP6091) was mapped to similar epitopes on the CH2 domain of human IgG1. Of the two anti-human IgG2 MAbs tested, HP6002 reacted with the CH2 of IgG2 while HP6014 bound to the CH1 domain. Both anti-human IgG3 MAbs (HP6047, HP6050) reacted with different regions of the IgG3 hinge. The anti-human IgG4 MAbs (HP6023, HP6025) bound to a similar epitope on the C-terminus of CH2 or the CH3 of human IgG4. The three exclusion antibodies (HP6019, HP6030, and HP6058) bound to different epitopes in the CH2 domain of three of four IgG subclasses. The domain mapping was confirmed by competitive inhibition expts. These results were used to select a group of IgG-reactive MAbs for construction of a poly-monoclonal anti-IgG capture and detection reagent that uniformly bound all four subclasses of human IgG. This study provides support for the use of engineered chimeric human chimeric antibodies as replacements for increasingly rare, purified human paraproteins in the specificity anal. of immunochem. reagents used in clin. and research labs. for the detection and quantitation of human antibodies. Moreover, these studies demonstrate how the MAbs can serve as effective probes for examining conformational differences among the four human IgG subclasses.

L18 ANSWER 19 OF 21 CAPLUS COPYRIGHT 2008 ACS on STN
1992:56938 Document No. 116:56938 Original Reference No. 116:9827a,9830a
Identification of epitopes recognized by a panel of six anti-human IgG2 monoclonal antibodies. Harada, Shigenori; Hata, Satoshi; Kosada, Yumi; Kondo, Eiji (Shionogi Inst. Med. Sci., Osaka, 556, Japan). Journal of Immunological Methods, 141(1), 89-96 (English) 1991. CODEN: JIMMBG. ISSN: 0022-1759.
AB Human IgG2 contains several subclass specific amino acid residues or deletions in the CH1 and CH2 domains and also in the hinge region. These substituted residues are the structural correlates for IgG2 specific epitopes. Since human IgG2 has different biol. properties from other subclasses, some IgG2 epitopes may be located in regions correlating with sites determining the biol. functions. Previously, three anti-IgG2 monoclonal antibodies (mAbs) with highly specific and interesting reactivities were produced using improved immunization protocols. However, it has been almost impossible to identify epitopes conventionally, because human IgG2 is so resistant to proteolysis that various proteolytic fragments could not be isolated. This study identified the epitopes recognized by anti-IgG2 mAbs by SDS-PAGE, Western blotting, amino acid sequence anal. and peptide/mAb binding ELISA, thus overcoming the need for fragment isolation. A panel of six anti-human IgG2 mAbs, including the current WHO/IUIS specificity stds. (HP6002, HP6008, HP6014) and 3 others (HG2-6A, HG2-30F, HG2-56F), reacted with distinct epitopes. The residues essential to expression of the epitopes recognized by the mAbs were: Pro234, Val235 and Val309 for GH2-56F, HG2-30F and HP6008, resp. HP6014 reacted with the epitope expressed by Thr214 and its neighboring residues. HG2-6A was reactive with the hinge region, and HP6002 was assumed to be directed against discontinuous epitopes requiring intact Fc for expression. Through this studies, the pepsin and papain cleavage sites of human IgG2 were also clarified.

L18 ANSWER 20 OF 21 CAPLUS COPYRIGHT 2008 ACS on STN
1990:511693 Document No. 113:111693 Original Reference No. 113:18841a,18844a

Serum half-life and tumor localization of a chimeric antibody deleted of the CH2 domain and directed against the disialoganglioside GD2. Mueller, Barbara M.; Reisfeld, Ralph A.; Gillies, Stephen D. (Dep. Immunol., Res. Inst. Scripps Clin., La Jolla, CA, 92037, USA). Proceedings of the National Academy of Sciences of the United States of America, 87(15), 5702-5 (English) 1990. CODEN: PNASA6. ISSN: 0027-8424.

AB A chimeric human/mouse antibody was engineered to the tumor-associated antigen ganglioside GD2, with the aim of decreasing its serum half-life, maintaining its full antigen-binding capacity, and deleting its effector functions, thus making it a potentially useful reagent for the radioimaging of tumors. To this end, the constant region of the human γ1 chain was mutated by deleting the second domain (CH2). The CH2-deleted antibody (ch14.18-ΔCH2) was cleared from the blood of athymic (nu/nu) mice bearing human melanoma tumors with the same kinetics as human IgG F(ab')2. At a β t1/2 of 12 h, 0.9% of the injected dose of 125-I-labeled ch14.18-ΔCH2 was found per mL of blood 24 h after i.v. injection. In biodistribution expts., 125I-labeled ch14.18-ΔCH2 targeted specifically to melanoma xenografts, achieving optimal tumor-to-tissue ratios 12-16 h after i.v. injection. Ch14.18-ΔCH2 was localized to the melanoma tumors more rapidly and with better localization ratios than the intact chimeric antibody ch14.18. Sixteen h after i.v. injection, the tumor-to-blood and tumor-to-liver ratios of ch14.18-ΔCH2 were 5 and 12, resp., while optimal localization ratios obtained for ch14.18 were 1 and 5, resp., but 96 h after injection. A reagent such as ch14.18-ΔCH2 should be useful for radioimmunodetection of human tumors because of reduced immunogenicity, increased targeting specificity,, and rapid clearance from circulation.

L18 ANSWER 21 OF 21 CAPLUS COPYRIGHT 2008 ACS on STN

1990:476476 Document No. 113:76476 Original Reference No. 113:12937a,12940a Antibodies having modified carbohydrate content and methods of preparation and use. Morrison, Sherie L.; Oi, Vernon T.; Hinton, Paul R. (Columbia University, USA; Becton, Dickinson and Co.). Eur. Pat. Appl. EP 359096 A1 19900321, 23 pp. DESIGNATED STATES: R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1989-116368 19890905. PRIORITY: US 1988-244744 19880915.

AB A method of altering the affinity of an antibody for the antigen to which it is directed comprises introducing into the variable region of the antibody a carbohydrate recognition site under conditions such that a carbohydrate binds to the site. The carbohydrate content is also modified by deleting from a constant region of the antibody a carbohydrate recognition site which naturally occurs in the constant region. The antibodies can be labeled, attached to a solid support, or conjugated with therapeutic ligands for use in anal., affinity chromatog., and therapy. The carbohydrate site in the CH2 domain of human IgG subclasses was deleted by site-directed mutagenesis of the DNA encoding the IgGs. The resultant antibodies had decreased ability to bind Fc receptors and to activate complement.

=> s chimeric CH2 domain
L21 0 CHIMERIC CH2 DOMAIN

=> s immunoglobulin
L22 1025078 IMMUNOGLOBULIN

=> s l22 and Fc
L23 59069 L22 AND FC

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=> s l23 and effector function
L24       1564 L23 AND EFFECTOR FUNCTION

=> s l24 and CH2 domain
L25         73 L24 AND CH2 DOMAIN

=> s l25 and domain swapping
L26          4 L25 AND DOMAIN SWAPPING

=> dup remove l26
PROCESSING COMPLETED FOR L26
L27          1 DUP REMOVE L26 (3 DUPLICATES REMOVED)

=> d l27 cbib abs

L27  ANSWER 1 OF 1     MEDLINE on STN                    DUPLICATE 1
2006577351.    PubMed ID: 16797726.   Molecular aspects of human FcgammaR
     interactions with IgG: functional and therapeutic consequences. Siberil
     Sophie; Dutertre Charles-Antoine; Boix Charlotte; Bonnin Emmanuelle; Menez
     Renee; Stura Enrico; Jorieux Sylvie; Fridman Wolf-Herman; Teillaud
     Jean-Luc. (Unite INSERM 255, IFR58, Universite Rene Descartes-Paris 5,
     Universite Pierre et Marie Curie-Paris 6, Centre de Recherches
     Biomedicales des Cordeliers, Paris, France. ) Immunology letters, (2006
     Aug 15) Vol. 106, No. 2, pp. 111-8.  Electronic Publication: 2006-06-12.
     Ref: 56. Journal code: 7910006. ISSN: 0165-2478. Pub. country:
     Netherlands. Language: English.
AB   The binding of IgG antibodies to receptors for the Fc region of
     IgG (FcgammaR) is a critical step for the initiation and/or the control of
     effector immune functions once immune complexes have been formed.
     Site-directed and random mutagenesis as well as domain-
     swapping, NMR and X-ray cristallography have made it possible to
     get detailed insights in the molecular mechanisms that govern IgG/FcgammaR
     interactions and to define some of the structural determinants that impact
     IgG binding to the various FcgammaR.  It has demonstrated the role of
     particular stretches and individual residues located in the lower hinge
     region of the CH2 domain and in the CH2 and CH3
     domains of the Fc region.  The importance of the sugar
     components linked to asparagine 297 in the binding properties of IgG1, the
     human IgG isotype the most widely used in antibody-based therapies, has
     been also highlighted.  These data have led to the engineering of a new
     generation of monoclonal antibodies for therapeutic use with optimized
     effector functions.


=> s l25 and substitution
L28         12 L25 AND SUBSTITUTION

=> dup remove l28
PROCESSING COMPLETED FOR L28
L29          7 DUP REMOVE L28 (5 DUPLICATES REMOVED)

=> d l29 1-7 cbib abs

L29  ANSWER 1 OF 7  SCISEARCH  COPYRIGHT (c) 2008 The Thomson Corporation  on
     STN
2008:442063   The Genuine Article (R) Number: 278CG. Structural
     characterization of a mutated, ADCC-enhanced human Fc fragment.
     Oganesyan, Vaheh; Damschroder, Melissa M.; Leach, William; Wu, Herren
     (Reprint); Dall'Acqua, William F.. Medimmune Inc, Dept Antibody Discovery
     & Prot Engn, 1 Medimmune Way, Gaithersburg, MD 20878 USA (Reprint);
     Medimmune Inc, Dept Antibody Discovery & Prot Engn, Gaithersburg, MD 20878
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USA; Medimmune Inc, Dept Proc Biochem, Gaithersburg, MD 20878 USA. wuh@medimmune.com; dallacquaw@medimmune.com. MOLECULAR IMMUNOLOGY (APR 2008) Vol. 45, No. 7, pp. 1872-1882. ISSN: 0161-5890. Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We report here the three-dimensional structure of a human Fc fragment engineered for enhanced antibody dependent cell mediated cytotoxicity (ADCC). The triple mutation S239D/A330L/I332E ('3M') was introduced into the C(H)2 portion of a human immunoglobulin G1 (IgG1) Fc. These three substitutions typically result in an about 10-100-fold increase in human IgG1 binding to human Fc gamma RIIIA (CD 16). The recombinantly produced Fc/3M fragment was crystallized and its structure solved at a resolution of 2.5 angstrom using molecular replacement. No dramatic structural changes were observed in Fc/3M when compared with unmutated human Fc fragments. However, we found that the relative positions of its CH2 domains allowed for an unusually 'open' conformation of the entire fragment. Although this particular structural feature could be due to crystallization artifacts or intrinsic variability, we propose that molecular mechanisms at the basis of the enhanced interaction between Fc/3M and CD16 could include enhanced Fc openness as well as the introduction of additional hydrophobic contacts, hydrogen bonds and/or electrostatic interactions at the corresponding interface. The existence of a more pronounced cleft between the two Fc chains as well as of repulsive, electrostatic intra-chain interactions may also account in part for the decreased thermostability of both Fc /3M and a 3M-modified humanized anti-human EphA2 IgG1 when compared with their respective unmutated counterparts. (C) 2007 Elsevier Ltd. All rights reserved.

L29 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2008 ACS on STN
2005:451416 Document No. 143:6282 Neonatal antibodies and Fc receptor-binding or Fc-containing polypeptide variants for treating cancer, immune or autoimmune disease and inflammation. Farrington, Graham K.; Lugovskoy, Alexey Alexandrovic; Eldredge, John K.; Garber, Ellen (Biogen Idec MA Inc., USA). PCT Int. Appl. WO 2005047327 A2 20050526, 168 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-US37929 20041112. PRIORITY: US 2003-519744P 20031112; US 2003-519743P 20031112; US 2003-519733P 20031112.

AB The compns. and methods of the present invention are based, in part, on our discovery that an effector function mediated by an Fc-containing polypeptide can be altered by modifying one or more amino acid residues within the polypeptide (by, for example, electrostatic optimization). The polypeptides that can be generated according to the methods of the invention are highly variable, and they can include antibodies and fusion proteins that contain an Fc region or a biol. active portion thereof. Thus, altered humanized IgG1 monoclonal antibody hCBE11, FcRn-Fc fusion protein and FcRn-huM4Fc fusion protein were prepared and tested.

L29 ANSWER 3 OF 7 MEDLINE on STN DUPLICATE 1
95309977. PubMed ID: 7540592. Activation of effector functions by immune complexes of mouse IgG2a with isotype-specific

autoantibodies. Rajnavolgyi E; Fazekas G; Lund J; Daeron M; Teillaud J L; Jefferis R; Fridman W H; Gergely J. (Department of Immunology, L. Eotvos University, God, Hungary.) Immunology, (1995 Apr) Vol. 84, No. 4, pp. 645-52. Journal code: 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Analysis of five monoclonal autoantibodies, rheumatoid factors produced by hybridomas generated from spleen cells of BALB/c mice repeatedly infected with A/PR/8/34 human influenza A virus, revealed that they recognized distinct but spatially related epitopes. The differing isoallotypic specificity of the IgM and IgA monoclonal antibodies correlated with the presence of Ile258 and Ala305, respectively. Although these data suggest that the epitopes recognized are within the CH2 domain , all antibodies failed to inhibit IgG antigen reactivity with Staphylococcus aureus protein A (SpA), C1q, mouse C3, human Fc gamma RI or mouse Fc gamma RII, activities known to be predominantly determined by CH2 domain structures. Reactivity of the IgA antibody, Z34, with IgG2b allowed further specificity studies using a panel of 26 mutant IgG2b proteins, each having single amino acid replacements over the surface of the CH2 domain. The only substitution that affected Z34 reactivity was Asn/Ala297, which destroyed the glycosylation sequon, resulting in secretion of an aglycosylated IgG molecule. The epitope recognized by Z34 therefore seems to be located outside of the Fc gamma R and C1q binding sites, but to be dependent on the presence of carbohydrate for expression. In contrast to the binding studies, complement activation by aggregated IgG2a, through classical or alternative pathways, was inhibited by the presence of autoantibodies. The functional significance of isotype-specific autoantibody in immune regulation is discussed.

L29 ANSWER 4 OF 7 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

1992:127840 The Genuine Article (R) Number: HF032. BLOOD CLEARANCE IN THE RAT OF A RECOMBINANT MOUSE MONOCLONAL-ANTIBODY LACKING THE N-LINKED OLIGOSACCHARIDE SIDE-CHAINS OF THE CH2 DOMAINS. WAWRZYNCZAK E J (Reprint); CUMBER A J; PARNELL G D; JONES P T; WINTER G. INST CANC RES, MED SECT, DRUG TARGETING LAB, SUTTON SM2 5NG, SURREY, ENGLAND (Reprint); MRC, MOLEC BIOL LAB, CAMBRIDGE CB2 2QH, ENGLAND. MOLECULAR IMMUNOLOGY (FEB 1992) Vol. 29, No. 2, pp. 213-220. ISSN: 0161-5890. Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, ENGLAND OX5 1GB. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB The serum half-lives of a wild-type recombinant mouse monoclonal antibody of the IgG2b isotype and a mutant antibody differing from the wild-type antibody by a single amino acid substitution introduced into the C(H)2 domain, the replacement of Asn 297 by Ala to delete the conserved site of heavy chain glycosylation, were determined in the rat. The biological half-life of the aglycosyl Asn 297-Ala mutant recombinant antibody (4.8 days) was significantly shorter than that of the normally glycosylated wild-type antibody (7.4 days) by enzyme immunoassay. A similar difference between the biological half-lives of I-125-labelled aglycosyl and wild-type antibodies (2.9 and 4.0 days, respectively) was determined by gamma counting. Analysis of serum samples demonstrated that both recombinant antibodies were present in the circulation predominantly as intact monomeric IgG and revealed no differences that could account for the more rapid elimination of the aglycosyl antibody. The results of this investigation indicate that the carbohydrate residues contribute only in part to the survival of IgG in vivo and suggest that the diminished half-life of the aglycosyl antibody is due to increased catabolism in the extravascular tissues.

L29 ANSWER 5 OF 7 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

1991:313325 The Genuine Article (R) Number: FN859. THE BINDING-AFFINITY OF HUMAN-IGG FOR ITS HIGH-AFFINITY FC RECEPTOR IS DETERMINED BY MULTIPLE AMINO-ACIDS IN THE CH2 DOMAIN AND IS MODULATED BY THE HINGE REGION. CANFIELD S M (Reprint); MORRISON S L. COLUMBIA UNIV COLL PHYS & SURG, DEPT MICROBIOL, NEW YORK, NY 10032 (Reprint); UNIV CALIF LOS ANGELES, DEPT MICROBIOL & MOLEC GENET, LOS ANGELES, CA 90024; UNIV CALIF LOS ANGELES, INST MOLEC BIOL, LOS ANGELES, CA 90024. JOURNAL OF EXPERIMENTAL MEDICINE (1 JUN 1991) Vol. 173, No. 6, pp. 1483-1491. ISSN: 0022-1007. Publisher: ROCKEFELLER UNIV PRESS, 222 E 70TH STREET, NEW YORK, NY 10021. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB A family of chimeric immunoglobulins (Igs) bearing the murine variable region directed against the hapten dansyl linked to human IgG1, -2, -3, and -4 has been characterized with respect to binding to the human high affinity Fc-gamma-receptor, Fc-gamma-R(I). Chimeric IgG1 and -3 have the highest affinity association (K(a) = 10(9) M-1), IgG4 is 10-fold reduced from this level, and IgG2 displays no detectable binding. A series of genetic manipulations was undertaken in which domains from the strongly binding subclass IgG3 were exchanged with domains from the nonbinding subclass IgG2. The subclass of the C(H)2 domain was found to be critical for determining IgG receptor affinity. In addition, the hinge region was found to modulate the affinity of the IgG for Fc-gamma-R(I), possibly by determining accessibility of Fc-gamma-R(I) to the binding site on Fc. A series of amino acid substitutions were engineered into the C(H)2 domain of IgG3 and IgG4 at sites considered potentially important to Fc receptor binding based on homology comparisons of binding and nonbinding IgG subclasses. Characterization of these mutants has revealed the importance for Fc-gamma-R(I) association of two regions of the genetic C(H)2 domain separated in primary structure by nearly 100 residues. The first of these is the hinge-link or lower hinge region, in which two residues, Leu(234) and Leu(235) in IgG1 and -3, are critical to high affinity binding. Substitution at either of these sites reduces the IgG association constant by 10-100-fold. The second region that appears to contribute to receptor binding is in a hinge-proximal bend between two beta-strands within the C(H)2 domain, specifically, Pro(331) in IgG1 and -3. As a result of beta-sheet formation within this domain, this residue lies within 11 angstrom of the hinge-link region. Substitution at this site reduces the Fc receptor association constant by 10-fold.

L29 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2008 ACS on STN

1989:207251 Document No. 110:207251 Original Reference No. 110:34286h,34287a Altered antibodies having altered effector functions and their preparation. Winter, Gregory Paul; Duncan, Alexander Robert; Burton, Dennis Raymond (Medical Research Council, UK). PCT Int. Appl. WO 8807089 A1 19880922, 42 pp. DESIGNATED STATES: W: AU, GB, JP, US; RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1988-GB211 19880318. PRIORITY: GB 1987-6425 19870318; GB 1987-18897 19870810; GB 1987-28042 19871201.

AB Antibodies (Ab) with altered binding affinity for effectors such as the Clq component of the complement system are prepared by replacing amino acid residues of the CH2 region using genetic engineering techniques. Human Cγ3 genes having a mutation, i.e. 234-leucine to alanine, 235-leuciner to glutamine, 236-glycine to alanine, and 237-glycine to alanine, resp., were constructed and cloned into expression vector pSBgpt after linking with the gene encoding the variable domain of the B18 antibody (Ab). The binding affinity I50 (the concentration of IgG3 at which the

fractional binding of 125I-labeled pooled human IgG is 0.5) to Fc γ R1 receptor on U937 cells of the recombinant mutants, i.e. [234-Ala]-IgG3, [235-Glu]-IgG3, [236-Ala]-IgG3, and [237-Ala]-IgG3 were 4 + 10-8, >10-6, 3 + 10-8, and 3 + 10-7M, resp., vs. 10-8M of the control using the wild-type IgG.

L29 ANSWER 7 OF 7 MEDLINE on STN DUPLICATE 2
89078461. PubMed ID: 3060362. Complement activation is not required for IgG-mediated suppression of the antibody response. Heyman B; Wiersma E; Nose M. (Department of Immunology, Uppsala University, Sweden.) European journal of immunology, (1988 Nov) Vol. 18, No. 11, pp. 1739-43. Journal code: 1273201. ISSN: 0014-2980. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.
AB Feedback suppression of the antibody response by IgG is known to be dependent on intact Fc regions. However, it is not clear which of the Fc-mediated effector functions is required. In the present report we have studied whether ability or inability of the IgG antibodies to activate the complement system was of consequence for their immunosuppressive effect. First, a monoclonal IgG1-anti-2,4,6-trinitrophenyl (TNP) antibody, unable to activate complement via the classical or alternate pathway, was shown to be able to inhibit more than 90% of the in vivo sheep erythrocyte-specific antibody response in mice when TNP coupled to sheep erythrocytes was used as antigen. Second, we investigated the immunosuppressive ability of a non-complement-activating mutant IgG2a-anti-TNP monoclonal antibody. The mutant differs from the wild type by a single amino acid substitution in the CH2 domain leading to inability to fix complement factor Clq. However, the mutant has the same affinity for antigen and the same Fc receptor-binding capacity as the wild type antibody. It is demonstrated that the mutant was as efficient as the wild type antibody in inhibiting an in vitro antibody response to TNP-coupled sheep erythrocytes. These findings confirm the non-determinant specificity and Fc dependence of IgG-mediated suppression, and show that the Fc-mediated effector mechanism is independent of complement activation. The results instead suggest binding to Fc receptors as a necessary step in feedback immunosuppression and favor inactivation of B cells by cross-linking of Fc and antigen receptors on their surface rather than elimination of antigen by complement-dependent phagocytosis as the effector mechanism.

=> s l23 and EU numbering
L30 37 L23 AND EU NUMBERING

=> s l30 and residues 231-340
L31 0 L30 AND RESIDUES 231-340

=> s l30 and substitution
L32 10 L30 AND SUBSTITUTION

=> dup remove l32
PROCESSING COMPLETED FOR L32
L33 4 DUP REMOVE L32 (6 DUPLICATES REMOVED)

=> d l33 1-4 cbib abs

L33 ANSWER 1 OF 4 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN
2007596137 EMBASE Solution conformation of wild-type and mutant IgG3 and IgG4 immunoglobulins using crystallohydrodynamics: Possible implications for complement activation.

Lu, Yanling; Harding, Stephen E. (correspondence); Longman, Emma; Davis, Kenneth G.. National Centre for Macromolecular Hydrodynamics, University of Nottingham, Sutton Bonington, United Kingdom. steve.harding@nottingham.ac.uk. Michaelsen, Terje E.. Norwegian Institute of Public Health, Oslo, Norway. Michaelsen, Terje E.. Institute of Pharmacy, University of Oslo, Blindern, Oslo, Norway. Ortega, Alvaro; Garcia De La Torre, Jose. Departamento de Quimica Fisica, Universidad de Murcia, Murcia, Spain. Grossmann, J. Gunter. Science and Technology Facilities Council, Daresbury Laboratory, Daresbury Science and Innovation Campus, Warrington, Cheshire, United Kingdom. Sandlie, Inger. Institute of Molecular Bioscience, University of Oslo, Blindern, Oslo, Norway.
Biophysical Journal Vol. 93, No. 11, pp. 3733-3744 1 Dec 2007.
Refs: 68.
ISSN: 0006-3495. CODEN: BIOJAU
Pub. Country: United States. Language: English. Summary Language: English.
Entered STN: 20071218. Last Updated on STN: 20071221

AB We have employed the recently described crystallohydrodynamic approach to compare the time-averaged domain orientation of human chimeric IgG3wt (wild-type) and IgG4wt as well as two hinge mutants of IgG3 and an IgG4S331P (mutation from serine to proline at position 331, EU numbering) mutant of IgG4. The approach involves combination of the known shape of the Fab and Fc regions from crystallography with hydrodynamic data for the Fab and Fc fragments and hydrodynamic and small angle x-ray scattering data for the intact IgG structures. In this way, ad hoc assumptions over hydration can be avoided and model degeneracy (uniqueness problems) can be minimized. The best fit model for the solution structure of IgG3wt demonstrated that the Fab regions are directed away from the plane of the Fc region and with a long extended hinge region in between. The best fit model of the IgG3m15 mutant with a short hinge (and enhanced complement activation activity) showed a more open, but asymmetric structure. The IgG3HM5 mutant devoid of a hinge region (and also devoid of complement-activation activity) could not be distinguished at the low-resolution level from the structure of the enhanced complement-activating mutant IgG3m15. The lack of inter-heavy-chain disulphide bond rather than a significantly different domain orientation may be the reason for the lack of complement-activating activity of the IgG3HM5 mutant. With IgG4, there are significant and interesting conformational differences between the wild-type IgG4, which shows a symmetric structure, and the IgG4S331P mutant, which shows a highly asymmetric structure. This structural difference may explain the ability of the IgG4S331P mutant to activate complement in stark contrast to the wild-type IgG4 molecule which is devoid of this activity. .COPYRGT. 2007 by the Biophysical Society.

L33 ANSWER 2 OF 4 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN DUPLICATE 1
1996013115 EMBASE Improved biodistribution, tumor targeting, and reduced immunogenicity in mice with a γ4 variant of Campath-1H.
Hutchins, Jeff T.; Kull Jr., Frederick C. (correspondence); Bynum, Jane; Knick, Vincent C.. Division of Cell Biology, Wellcome Research Laboratories, Research Triangle Park, NC 27709, United States. Ray, Paul. Division of Biochemistry, Wellcome Research Laboratories, Research Triangle Park, NC 27709, United States. Thurmond, Linda M.. Div. of Pharmacokin. and Drug Metab., Wellcome Research Laboratories, Research Triangle Park, NC 27709, United States. Hutchins, Jeff T.; Kull Jr., Frederick C. (correspondence); Bynum, Jane; Knick, Vincent C.; Thurmond, Linda M.. Glaxo Wellcome Co., Research Triangle Park, NC 27709, United States.
Proceedings of the National Academy of Sciences of the United States of America Vol. 92, No. 26, pp. 11980-11984 19 Dec 1995.
Refs: 32.

ISSN: 0027-8424. CODEN: PNASA6
Pub. Country: United States. Language: English. Summary Language: English.
Entered STN: 960130. Last Updated on STN: 960130

AB Radiolabeled antibodies have shown promise for the treatment of lymphoma and for solid tumor targeting. Campath-1H is a humanized monoclonal antibody that reacts with the CD52 antigen present on human lymphoid and myeloid cells. Campath-1H is a γ1 (G1) isotype that induces lymphopenia via an Fc- mediated mechanism(s). Isotype switches were engineered, and the resulting antibodies were expressed in NS0 mouse myeloma cells and biosynthetically radiolabeled with [(35)S]methionine. The forms included G1, G4, and a G4 variant that contained alanine substitutions at (EU numbering) Leu-235, Gly-237, and Glu-318. All isotypes bound antigen equivalently as assessed by target cell binding in vitro. The G4 variant had a greatly reduced capacity to interact with Fc receptor by virtue of reduced binding to THP-1 human myeloid cells and by a 1000-fold increase in EC(50) to intermediate antibody- dependent cellular cytotoxicity. The pharmacokinetics of the isotypes were compared in CD-1 (nu/nu) mice bearing an experimental antigen-expressing tumor. The plasma half-life and tumor uptake were increased for the G4 variant. The G4 variant showed significantly less spleen, liver, and bone uptake but similar uptake in the lung, kidney, and stomach and lower tissue- to-blood ratios. Immunogenicity was assessed after repeated monthly administrations of unlabeled antibody in BALB/c mice. A 50% reduction in the incidence of anti-globulin response was observed for the G4 variant. These properties suggest that antibodies with reduced Fc receptor interaction merit additional study as potential targeting vehicles relative to other isotypes for radioimmunotherapy or situations where diminished normal tissue binding contributes to efficacy.

L33 ANSWER 3 OF 4 MEDLINE on STN DUPLICATE 2

94043382. PubMed ID: 8227075. Identification of a secondary Fc gamma RI binding site within a genetically engineered human IgG antibody. Chappel M S; Isenman D E; Oomen R; Xu Y Y; Klein M H. (Department of Immunology, University of Toronto, Ontario, Canada.) The Journal of biological chemistry, (1993 Nov 25) Vol. 268, No. 33, pp. 25124-31. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Although human IgG2 is not cytophilic, we have shown previously that an IgG2 antibody expressing the sequence PLLGG (underline = substitution) spanning CH2 domain residues 233-237 (Eu numbering) displayed IgG1-like Fc gamma RI binding activity. In contrast, IgG1 PLLGG exhibited 3-fold less affinity, whereas IgG2 ELLGG was 3-fold more active than native IgG1. These results suggested that additional site(s) conferred enhanced binding properties to the engineered, cytophilic IgG2 variant. These sites were shown to reside in the IgG2 CH2 domain, since the IgG1 CH2 module did not have enhanced activity in a panel of hybrid IgG1/IgG2 antibodies. To map these sites further, human IgG1 and IgG2 constant region gene segments were modified to allow reciprocal COOH-terminal half segment exchanges of CH2 exons. These were cloned into a pSV2neo expression vector bearing a rearranged MOPC 315 heavy chain variable region gene and transfected into a MOPC 315 heavy chain deletion mutant. The dinitrophenol affinity-purified IgGs were radiolabeled and assessed for Fc gamma RI binding activity in direct binding assays using U937 cells. The COOH terminus of the IgG2 CH2 domain was found to contain accessory site(s) since it enhanced the binding properties of both IgG1 PLLGG and native IgG1. In contrast, grafting of the COOH terminus of the IgG1 CH2 domain onto IgG2 PLLGG and IgG2 ELLGG diminished their cytophilic activity. The amino acid responsible for the enhancing properties of the COOH terminus of the IgG2 CH2 domain was shown to be threonine 339, since IgG1 PLLGG/Thr339

displayed increased Fc gamma RI binding affinity. Kinetics studies revealed that this is accomplished through an increase in the forward rate constant of the IgG-Fc gamma RI interaction.

L33 ANSWER 4 OF 4 MEDLINE on STN DUPLICATE 3
90041556. PubMed ID: 2810890. An amino acid substitution determining G1m(x) allotypic marker. Ito S; Suzuki K; Miyazaki T; Matsumoto H. Nihon hoigaku zasshi = The Japanese journal of legal medicine, (1989 Apr) Vol. 43, No. 2, pp. 155-60. Journal code: 0413715. ISSN: 0047-1887. Pub. country: Japan. Language: Japanese.
AB We confirmed the amino acid substitution responsible for G1m(x) allotypic marker, which was estimated by Cook & Steinberg (Mol. Immunol. 16:555-558 (1979), by primary structure analysis of the C-terminal BrCN peptide from normal human IgG1 carrying G1m(x+) or G1m(x-). The results indicate that IgG1 carrying G1m(x) have glycine instead of alanine at position 431 (Eu numbering). And we discussed the epitope on tertiary structure of IgG1-Fc fragment.

=> s l22 and EU numbering
L34 51 L22 AND EU NUMBERING

=> s l34 and Kabat
L35 0 L34 AND KABAT

=> s l34 and (chimeric)adj(CD2)adj(domain)
MISSING OPERATOR CHIMERIC)ADJ
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l134 and chimeric CD2 domain
L134 NOT FOUND
The L-number entered could not be found. To see the definition
of L-numbers, enter DISPLAY HISTORY at an arrow prompt (=>).

=> s l34 and chimeric
L36 6 L34 AND CHIMERIC

=> dup remove l36
PROCESSING COMPLETED FOR L36
L37 2 DUP REMOVE L36 (4 DUPLICATES REMOVED)

=> d l37 1-2 cbib abs

L37 ANSWER 1 OF 2 MEDLINE on STN DUPLICATE 1
2007674618. PubMed ID: 17704171. Solution conformation of wild-type and mutant IgG3 and IgG4 immunoglobulins using crystallohydrodynamics: possible implications for complement activation. Lu Yanling; Harding Stephen E; Michaelsen Terje E; Longman Emma; Davis Kenneth G; Ortega Alvaro; Grossmann J Gunter; Sandlie Inger; Garcia de la Torre Jose. (National Centre for Macromolecular Hydrodynamics, University of Nottingham, Sutton Bonington, England.) Biophysical journal, (2007 Dec 1) Vol. 93, No. 11, pp. 3733-44. Electronic Publication: 2007-08-17. Journal code: 0370626. E-ISSN: 1542-0086. Pub. country: United States. Language: English.
AB We have employed the recently described crystallohydrodynamic approach to compare the time-averaged domain orientation of human chimeric IgG3wt (wild-type) and IgG4wt as well as two hinge mutants of IgG3 and an IgG4S331P (mutation from serine to proline at position 331, EU numbering) mutant of IgG4. The approach involves combination of the known shape of the Fab and Fc regions from crystallography with

hydrodynamic data for the Fab and Fc fragments and hydrodynamic and small angle x-ray scattering data for the intact IgG structures. In this way, ad hoc assumptions over hydration can be avoided and model degeneracy (uniqueness problems) can be minimized. The best fit model for the solution structure of IgG3wt demonstrated that the Fab regions are directed away from the plane of the Fc region and with a long extended hinge region in between. The best fit model of the IgG3m15 mutant with a short hinge (and enhanced complement activation activity) showed a more open, but asymmetric structure. The IgG3HM5 mutant devoid of a hinge region (and also devoid of complement-activation activity) could not be distinguished at the low-resolution level from the structure of the enhanced complement-activating mutant IgG3m15. The lack of inter-heavy-chain disulphide bond rather than a significantly different domain orientation may be the reason for the lack of complement-activating activity of the IgG3HM5 mutant. With IgG4, there are significant and interesting conformational differences between the wild-type IgG4, which shows a symmetric structure, and the IgG4S331P mutant, which shows a highly asymmetric structure. This structural difference may explain the ability of the IgG4S331P mutant to activate complement in stark contrast to the wild-type IgG4 molecule which is devoid of this activity.

L37 ANSWER 2 OF 2 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

1993:378088 The Genuine Article (R) Number: LH139. ACTIVATION OF COMPLEMENT BY AN IGG MOLECULE WITHOUT A GENETIC HINGE. BREKKE O H (Reprint); MICHAELSEN T E; SANDIN R; SANDLIE I. UNIV OSLO, DEPT BIOL, POB 1050, N-0316 BLINDERN, NORWAY; NATL INST PUBL HLTH, N-0462 OSLO, NORWAY. NATURE (17 JUN 1993) Vol. 363, No. 6430, pp. 628-630. ISSN: 0028-0836. Publisher: MACMILLAN MAGAZINES LTD, PORTERS SOUTH, 4 CRINAN ST, LONDON, ENGLAND N1 9XW. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB THE hinge region links the two Fab arms to the Fc portion of the IgG molecule. It mediates flexibility to the molecule and serves as a connecting structure between the two heavy chains. In addition it provides space between the Fab and Fc parts. All three properties have been proposed to be important for the ability of IgG to initiate complement activation leading to complement-mediated cell lysis (CML)1. Here we report the construction of a hinge-deleted mouse-human chimaeric IgG3 molecule with specificity for the hapten NIP (3-iodo-4-hydroxy-5-nitrophenacetyl), HM-1. HM-1 lacks the genetic hinge, but has an introduced cysteine between Ala 231 (EU numbering) and Pro 232 in the lower hinge encoded by the C(H)2 exon. The introduced cysteine forms a disulphide bond between the two heavy chains of the molecule. In CML, HM-1 shows a greater activity than IgG3 wild type. This is the first time an IgG molecule without a genetic hinge has been found to be active in CML. We conclude that the hinge functioning as a spacer is not a prerequisite for complement activation. Rather, its major role seems to be to connect the heavy chains to each other in the amino-terminal part of C(H)2. Because HM-1 is expected to have low Fab-Fc flexibility, this molecular feature is probably of no importance for complement activation.

=> s antibod?
L38 3114457 ANTIBOD?

=> s l38 and RhD antigen
L39 146 L38 AND RHD ANTIGEN

=> s l39 and CH2 domain
L40 0 L39 AND CH2 DOMAIN

```
=> s l39 and substitution
L41          7 L39 AND SUBSTITUTION

=> s l41 and effector function
L42          0 L41 AND EFFECTOR FUNCTION

=> dup remove l42
L42 HAS NO ANSWERS

=> dup remove l41
PROCESSING COMPLETED FOR L41
L43           3 DUP REMOVE L41 (4 DUPLICATES REMOVED)

=> d l43 1-3 cbib abs

L43  ANSWER 1 OF 3  EMBASE  COPYRIGHT (c) 2008 Elsevier B.V. All rights
     reserved on STN
2004196362  EMBASE  [Rh complex R(0)(Har) and Rh protein D HAR - RhD
     antigen variant with the most atypical genotypic background and
     phenotypic expression]. Rh komplex R(0)(Har) a Rh protein D HAR -
     Variantni RhD antigen s "nejabnormalnejs im"
     molekularnim podkladem i fenotypovou expresi.
     Pisacka, M., Dr. (correspondence). Ref. Laborator pro Imunohematologii,
     Ustav Hematologie a Krevni Transfuze, U nemocnice 1, 128 20 Praha 2, Czech
     Republic. pisacka@uhkt.cz. Pisacka, M., Dr. (correspondence). Ref.
     Laborator pro Imunohematologii, Ustav Hematologie a Krevni Transfuse, U
     nemocnice 1, 128 20 Praha 2, Czech Republic. pisacka@uhkt.cz.
     Transfuze a Hematologie Dnes  Vol. 10, No. 1, pp. 8-12  2004.
     Refs: 14.
     ISSN: 1213-5763. CODEN: THDRAK
     Pub. Country: Czech Republic. Language: Czech. Summary Language: Czech;
     English.
     Entered STN: 20040520. Last Updated on STN: 20040520
AB   Rh system belongs to the most important blood group systems.  Its
     molecular basis are two highly homologous genes (RHD a RHCE) localized in
     closed proximity and in opposite orientation on the first chromozome
     (1p34-36).  Products of these genes are two proteins, RhD and RhCcEe,
     carrying the Rh antigens.  RhD protein is the most immunogenic antigen
     complex of the red cell membrane - RhD negative phenotype is characterized
     by its total absence - that is why the immune system of RhD- people is
     very sensitive to contact with RhD positive erythrocytes (frequent
     antibody production in pregnancy or after transfusion of RhD+ red
     cells).  Other antigenic differences (C+/-, c+/-, E+/-, e+/- aj.) on the
     other hand are represented by only one or few aminoacid
     substitutions in one protein and therefore are much less
     immunogenic.  The detection of RhD in cca 1-2% RhD positive samples is
     complicated by the abnormal expression of the protein, which can be
     divided into quantitative weakening (less protein copies on the membrane)
     and into qualitative variants.  First type (weak D) is based on RHD(weak)
     gene mutations which do not affect the extramembranous portion of the
     protein.  The second type (D variants) is based on RHD(variant) gene with
     alteration(s) resulting in the loss of one or more D epitopes - this
     feature is used the serologic characterisation of variants and is the
     cause of their clinical importance (carriers of D-epitope deficient
     phenotype are able to form antibodies against "missing" part of
     the protein when contacted with "normal" RhD positive red cells).  This
     article is describing one unique RhD variant - R(o)Har, which dramatically
     differs from other types due to its molecular background (presence of only
     one hybrid RHCE-RHD(5)-RHCE gene) as well as serologic characteristics
     (unusual negative reactions with most IgG anti-D in the antiglobulin
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test), causing frequent discrepancies and diagnostic complications.

L43 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 1
2001671407. PubMed ID: 11716963. Epitope mapping of four monoclonal antibodies specific for the human RhD antigen.
Nickerson Lise; Wiersma Erik J. (Cangene Corporation, 3403 American Drive, Ontario, L4V 1T4, Mississauga, Canada.. lnickerson@cangene.com) .
Immunology letters, (2002 Jan 1) Vol. 80, No. 1, pp. 33-9. Journal code: 7910006. ISSN: 0165-2478. Pub. country: Netherlands. Language: English.
AB RhD is a highly immunogenic erythrocyte membrane protein, implicated in hemolytic disease of the newborn and other hemolytic disorders. Anti-RhD antibodies are used in the treatment of such disease states. Six mutant forms of recombinant RhD were stably expressed in K562 cells, and these cells were used to investigate epitope specificities of four anti-RhD monoclonal antibodies (mAbs). Amino acid substitutions were made in the exofacial loops of RhD to the corresponding residues found in the related RhCE polypeptide; M169L/M170R and I172F in the third loop, F223V and E233Q in the fourth loop, and D350H and G353W/A354N in the sixth loop. Each mAb was found to have a unique fine specificity and recognized multiple distant sites within RhD. The mAbs also differed in how they recognized individual amino acids in the exofacial loops of RhD.

L43 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2008 ACS on STN
2000:242160 Document No. 133:29394 Weak D alleles express distinct phenotypes. Wagner, Franz F.; Frohmajer, Alexander; Ladewig, Birgit; Eicher, Nicole I.; Lonicer, Cornelie B.; Muller, Thomas H.; Siegel, Manfred H.; Flegel, Willy A. (Abteilung Transfusionsmedizin, Institut Ulm, Universitatsklinikum Ulm and DRK-Blutspendedienst Baden-Wurttemberg, Ulm, D-89081, Germany). Blood, 95(8), 2699-2708 (English) 2000. CODEN: BLOOAW. ISSN: 0006-4971. Publisher: American Society of Hematology.
AB The weak D phenotype is caused by many different RHD alleles encoding aberrant RhD proteins, raising the possibility of distinct serol. phenotypes and of anti-D immunizations in weak D. We reported 6 new RHD alleles, D category III type IV, DIM, and the weak D types 4.1, 4.2.1, 4.2.2, and 17. The immunohematol. features of 18 weak D types were examined by agglutination and flow cytometry with more than 50 monoclonal anti-D. The agglutination patterns of the partial D phenotypes DIM, DIII type IV, and DIV type III correlated well with the D epitope models, those of the weak D types showed no correlation. In flow cytometry, the weak D types displayed type-specific antigen densities between 70 and 4000 RhD antigens per cell and qual. distinct D antigens. A Rhesus D similarity index was devised to characterize the extent of qual. changes in aberrant D antigens and discriminated normal D from all tested partial D, including D category III. In some rare weak D types, the extent of the alterations was comparable to that found in partial Ds that were prone to anti-D immunization. Four of 6 case reports with anti-D in weak D represented auto-anti-D. We concluded that, in contrast to previous assumptions, most weak D types, including prevalent ones, carry altered D antigens. These observations are suggestive of a clin. relevant potential for anti-D immunizations in some, but not in the prevalent weak D types, and were used to derive an improved transfusion strategy in weak D patients.

=> s l38 and human platelet antigen
L44 528 L38 AND HUMAN PLATELET ANTIGEN

=> s l44 and reduced complement
L45 0 L44 AND REDUCED COMPLEMENT

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=> s 144 and reduced Fc function
L46           0 L44 AND REDUCED FC FUNCTION

=> s 144 and CH2 domain
L47           0 L44 AND CH2 DOMAIN

=> s 144 and CH2 substitution
L48           0 L44 AND CH2 SUBSTITUTION

=> s anti-CD52
L49         826 ANTI-CD52

=> s 149 and CH2 domain
L50           0 L49 AND CH2 DOMAIN

=> s 149 and Fc function
L51           0 L49 AND FC FUNCTION

=> s 149 and chimeric
L52          38 L49 AND CHIMERIC

=> s 152 and effector
L53           5 L52 AND EFFECTOR

=> dup remove 153
PROCESSING COMPLETED FOR L53
L54            2 DUP REMOVE L53 (3 DUPLICATES REMOVED)

=> d 154 1-2 cbib abs

L54  ANSWER 1 OF 2  EMBASE  COPYRIGHT (c) 2008 Elsevier B.V. All rights
     reserved on STN
2003267899  EMBASE  New monoclonal antibodies in renal transplantation.
     Vincenti, F. (correspondence). Kidney Transplant Service, University of
     California, San Francisco, CA, United States. vincentif@surgery.ucsf.edu.
     Vincenti, F. (correspondence). University of California, Kidney Transplant
     Service, 505 Parnassus Avenue, San Francisco, CA 941430780, United States.
     vincentif@surgery.ucsf.edu.
     Minerva Urologica e Nefrologica  Vol. 55, No. 1, pp. 57-66  Mar 2003.
     Refs: 50.
     ISSN: 0393-2249. CODEN: MUNEEF
     Pub. Country: Italy. Language: English. Summary Language: English;
     Italian.
     Entered STN: 20030724. Last Updated on STN: 20030724
AB   A decade of spectacular innovation in maintenance immunosuppression drugs
     has resulted in dramatic reductions in acute rejection and improvement in
     short and long term outcome after renal transplantation.  However the new
     drugs continue to lack specificity, many require frequent therapeutic drug
     monitoring and all are associated with acute and chronic toxicities.  The
     new biologic agents, monoclonal antibodies (chimeric, humanized,
     and fully human) and receptor-fusion proteins, lack immunogenicity, have
     long half-life and prolonged biologic effects, require intermittent
     administration and have minimal toxicity.  The specificity and selectively
     of the targets of the new biologic agents render them less toxic than the
     oral maintenance drugs and thus could possibly replace the maintenance
     drugs most associated with long-term toxicity such as the corticosteroids
     and the calcineurin inhibitors.  The recently introduced anti-interleukin
     2 receptor (IL-2R) monoclonal antibodies (mAbs) are the prototype of
     future biologic agents; selective, safe, and inducing prolonged biologic
     effects.  The IL-2R mAbs have been used with a variety of maintenance
     immunosuppression regimens double therapy with cyclosporine and
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prednisone, triple therapy with cyclosporine, azathioprine and prednisone and with newer regimens such as cyclosporine or tacrolimus, mycophenolate mofetil (MMF) and prednisone, and most recently with sirolimus, MMF and prednisone. The major thrust of the new biologics in clinical development is to block the co-stimulatory pathway. The first attempt at blockade of the CD40-CD154 with anti-CD154 mAbs was disappointing. Anti-CD 154 therapy was associated with thromboembolic events and acute rejection. Attempts at blocking the CD28-B7s (CD80-CD86) pathway are currently underway with the receptor fusion protein, LEA29Y a second generation CTL4Aig, and humanized mAbs to CD 80 and CD86. LFA1, an adhesion molecule that also participates in the co-stimulatory pathway, has also been targeted with a mAb that binds to the CD11a chain of LFA1. Efalizumab, a humanized anti-CD11a mAb, was shown in a phase I trial to be potentially effective in renal transplantation. A humanized anti-CD45 RB mAb is currently in pre-clinical studies and will likely be tested in a phase I trial of renal transplantation within 1 year. While excellent results with anti-CD45 RB mAbs have been published in experimental transplantation, the mechanism of action of anti-CD45 RB mAbs remains to be determined. Several antibodies that are currently approved for non-transplant indications are currently used in single center clinical trials in renal transplantation including Campath 1 H, a humanized anti-CD52 mAb, Rituxamab, an anti-CD20 chimeric mAb, and Infliximab an anti-TNFα chimeric mAb. In addition, several humanized mutagenized anti-CD3 mAbs, huOKT3(γ1), aglycosyl CD3 and HuM291 have been used in limited trials in renal transplantation but have yet to have a formal clinical development. Humanized mAbs and receptor fusion proteins offer the potential of providing renal transplant recipients with a novel algorithm for immunosuppression that relies on chronic intermittent intravenous administration of safe, non-toxic agents replacing oral drug therapy maintenance.

L54 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 1
2004097689. PubMed ID: 14988743. Monoclonal antibodies in human cancer. Mellstedt H. (Department of Oncology and Hematology, Karolinska Cancer Center, Karolinska Hospital, Stockholm, Sweden.) Drugs of today (Barcelona, Spain : 1998), (2003) Vol. 39 Suppl C, pp. 1-16. Ref: 56. Journal code: 101160518. ISSN: 1699-3993. Pub. country: Spain. Language: English.

AB Mouse, chimeric, humanized and human monoclonal antibodies (MABs) are all in use for treatment of human cancer. Unconjugated antibodies have a complex mechanism of action, dependent on the nature of the target structure. Antibodies can activate the immune system (antibody-dependent cellular cytotoxicity [ADCC], complement-dependent cytotoxicity [CDC], induction of tumor immunity [idiotype network]). ADCC appears to be one of the most important immune effector functions. Antibodies may also induce apoptosis, cell cycle arrest, inhibition of cell proliferation as well as angiogenesis and metastatic spread. For most antibodies there is no clear dose-response relationship in vivo. The effect of antibodies can be enhanced by combination with chemotherapy and/or by agents which activate the immune system. The best therapeutic effect may be obtained if MABs are used early in the course of the disease. Rituximab (anti-CD20) was the first registered MAB for the therapy of follicular lymphoma. Impressive results have been seen in combination with CHOP chemotherapy (cyclophosphamide, doxorubicin, vincristine and prednisone) in follicular and high-grade lymphomas. In other non-Hodgkin's lymphoma subtypes, promising results are also seen in combination with chemotherapy. Trastuzumab (anti-Her2) is a breakthrough in the treatment of breast cancer in combination with chemotherapeutic agents. This antibody is also in clinical testing for adjuvant treatment. Alemtuzumab (anti-CD52) has shown impressive results

both in refractory chronic lymphocytic leukemia and as up-front therapy. There are many other antibodies in late stages of testing for registration. Interesting MABs include cetuximab (anti-epidermal growth factor receptor [EGFR]), especially in combination with radiotherapy in head and neck cancer; ABX-EGF (anti-EGFR) in renal carcinoma; bevacizumab (anti-vascular endothelial growth factor) in several solid tumors. Antiepithelial cell adhesion molecule antibodies show promise in combination with chemotherapy in the adjuvant setting of colorectal carcinoma. It is estimated that about 20 antibodies will be in clinical use by the year 2010.

=> s modified effector function
L55 2 MODIFIED EFFECTOR FUNCTION

=> dup remove l55
PROCESSING COMPLETED FOR L55
L56 2 DUP REMOVE L55 (0 DUPLICATES REMOVED)

=> d l56 1-2 cbib abs

L56 ANSWER 1 OF 2 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
2004:307651 Document No.: PREV200400311240. CTLA4-immunoglobulin fusion proteins having modified effector functions and uses therefor. Gray, Gary S. [Inventor, Reprint Author]; Carson, Jerry [Inventor]; Javaherian, Kashi [Inventor]; Jellis, Cindy L. [Inventor]; Rennert, Paul D. [Inventor]; Silver, Sandra [Inventor]. ASSIGNEE: Repligen Corporation, Newton, MA, USA. Patent Info.: US 6750334 20040615. Official Gazette of the United States Patent and Trademark Office Patents, (June 15 2004) Vol. 1283, No. 3. http://www.uspto.gov/web/menu/patdata.html. e-file.
ISSN: 0098-1133 (ISSN print). Language: English.
AB CTLA4-immunoglobulin fusion proteins having modified immunoglobulin constant region-mediated effector functions, and nucleic acids encoding the fusion proteins, are described. The CTLA4-immunoglobulin fusion proteins comprise two components: a first peptide having a CTLA4 activity and a second peptide comprising an immunoglobulin constant region which is modified to reduce at least one constant region-mediated biological effector function relative to a CTLA4-IgG1 fusion protein. The nucleic acids of the invention can be integrated into various expression vectors, which in turn can direct the synthesis of the corresponding proteins in a variety of hosts, particularly eukaryotic cells. The CTLA4-immunoglobulin fusion proteins described herein can be administered to a subject to inhibit an interaction between a CTLA4 ligand (e.g., B7-1 and/or B7-2) on an antigen presenting cell and a receptor for the CTLA4 ligand (e.g., CD28 and/or CTLA4) on the surface of T cells to thereby suppress an immune response in the subject, for example to inhibit transplantation rejection, graft versus host disease or autoimmune responses.

L56 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2008 ACS on STN
1997:536915 Document No. 127:219551 Antibodies and immunoglobulin fusion proteins having modified effector functions and uses therefor. Gray, Gary S.; Carson, Jerry; Javaherian, Kashi; Jellis, Cindy L.; Rennert, Paul D.; Silver, Sandra (Repligen Corp., USA; Gray, Gary S.; Carson, Jerry; Javaherian, Kashi; Jellis, Cindy L.; Rennert, Paul D.; Silver, Sandra). PCT Int. Appl. WO 9728267 A1 19970807, 104 pp. DESIGNATED STATES: W: AU, CA, JP, US; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US1698 19970203. PRIORITY: US 1996-595590 19960202.
AB CTLA4-Ig fusion proteins having modified Ig constant region-mediated

effector functions, and nucleic acids encoding the fusion proteins, are described. The CTLA4-Ig fusion proteins comprise two components: a first peptide having a CTLA4 activity and a second peptide comprising an Ig constant region which is modified to reduce at least one constant region-mediated biol. effector function relative to a CTLA4-IgG1 fusion protein. The nucleic acids of the invention can be integrated into various expression vectors, which in turn can direct the synthesis of the corresponding proteins in a variety of hosts, particularly eukaryotic cells. The CTLA4-Ig fusion proteins described herein can be administered to a subject to inhibit an interaction between a CTLA4 ligand (e.g., B7-1 and/or B7-2) on an antigen presenting cell and a receptor for the CTLA4 ligand (e.g., CD28 and/or CTLA4) on the surface of T cells to thereby suppress an immune response in the subject, for example to inhibit transplantation rejection, graft vs. host disease or autoimmune responses.

=> s anti-RhD
L57 274 ANTI-RHD

=> s l57 and CH2 domain
L58 5 L57 AND CH2 DOMAIN

=> dup remove l58
PROCESSING COMPLETED FOR L58
L59 1 DUP REMOVE L58 (4 DUPLICATES REMOVED)

=> d l59 cbib abs

L59 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
1999388014. PubMed ID: 10458776. Recombinant human IgG molecules lacking Fcgamma receptor I binding and monocyte triggering activities. Armour K L; Clark M R; Hadley A G; Williamson L M. (Division of Immunology Department of Pathology, University of Cambridge, Cambridge, GB.) European journal of immunology, (1999 Aug) Vol. 29, No. 8, pp. 2613-24. Journal code: 1273201. ISSN: 0014-2980. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Subclasses of human IgG have a range of activity levels with different effector systems but each triggers at least one mechanism of cell destruction. We are aiming to engineer non-destructive human IgG constant regions for therapeutic applications where depletion of cells bearing the target antigen is undesirable. The attributes required are a lack of killing via Fcgamma receptors (R) and complement but retention of neonatal FcR binding to maintain placental transport and the prolonged half-life of IgG. Eight variants of human IgG constant regions were made with anti-RhD and CD52 specificities. The mutations, in one or two key regions of the CH2 domain, were restricted to incorporation of motifs from other subclasses to minimize potential immunogenicity. IgG2 residues at positions 233 - 236, substituted into IgG1 and IgG4, reduced binding to FcgammaRI by 10(4)-fold and eliminated the human monocyte response to antibody-sensitized red blood cells, resulting in antibodies which blocked the functions of active antibodies. If glycine 236, which is deleted in IgG2, was restored to the IgG1 and IgG4 mutants, low levels of activity were observed. Introduction of the IgG4 residues at positions 327, 330 and 331 of IgG1 and IgG2 had no effect on FcgammaRI binding but caused a small decrease in monocyte triggering.

=> s anti-HPA-1a
L60 433 ANTI-HPA-1A

=> s l60 and CH2 domain

```
L61           0 L60 AND CH2 DOMAIN

=> s l60 and reduced complement lysis
L62           0 L60 AND REDUCED COMPLEMENT LYSIS

=> s l60 and Fc receptor binding
L63           0 L60 AND FC RECEPTOR BINDING

=> s l60 and Fc receptor
L64           5 L60 AND FC RECEPTOR

=> s l64 and reduced binding
L65           0 L64 AND REDUCED BINDING

=> dup remove l64
PROCESSING COMPLETED FOR L64
L66            4 DUP REMOVE L64 (1 DUPLICATE REMOVED)

=> d l66 1-4 cbib abs

L66  ANSWER 1 OF 4  EMBASE  COPYRIGHT (c) 2008 Elsevier B.V. All rights
     reserved on STN
2003221972  EMBASE  The role of P-selectin in the immune destruction of
     platelets.
     Turner, Craig P. (correspondence); Hadley, Andrew G.. Bristol Inst. for
     Transfus. Sciences, Bristol, United Kingdom. craig.turner@nbs.nhs.uk.
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     craig.turner@nbs.nhs.uk.
     British Journal of Haematology  Vol. 121, No. 4, pp. 623-631  May 2003.
     Refs: 34.
     ISSN: 0007-1048. CODEN: BJHEAL
     Pub. Country: United Kingdom. Language: English. Summary Language:
     English.
     Entered STN: 20030619. Last Updated on STN: 20030619
AB   Antibody-mediated platelet destruction is a poorly understood process,
     although several lines of evidence suggest that Fcγ receptor
     (FcγR)-expressing splenic macrophages may be involved.  In this
     study, chemiluminescence (CL) was used to measure the in vitro metabolic
     response of human monocytes to platelets sensitized with a human
     immunoglobulin (Ig)G1 recombinant antihuman platelet antigen-1a (
     anti-HPA-1a) antibody (B2G1; P-hrIgG1).  CL
     responses were inhibited, but not abrogated, in the presence of 10
     μg/ml human IgG or murine IgG2a, suggesting that FcγRI was
     principally involved.  Experiments to determine the effect of Fab
     fragments to FcγRII found that CL responses to P-hrIgG1 were
     significantly enhanced, indicating that crosslinking of monocyte
     FcγRII by platelet-bound hIgG may modulate concomitant activation by
     FcγRI.  Several observations suggested that the CL responses to
     P-IgG were dependent on the activation of resting platelets during their
     co-culture with monocytes and their subsequent P-selectin-mediated
     adhesion.  First, the magnitude of the CL response was related to the
     level of P-selectin expression following platelet activation with
     α-thrombin.  Second, CL responses were inhibited in the presence of
     antibodies that block the binding of P-selectin to P-selectin glycoprotein
     ligand-1 but not when platelets were pretreated and then washed.  Third,
     the addition of anti-HPA-1a to monocytes
     from HPA-1a-negative donors preincubated with HPA-1a-positive platelets
     resulted in rapid CL responses.  Finally, PGI(2) inhibited the CL response
     to resting P-hrIgG1.  Thus, evidence is presented that the interaction of
     human monocytes with P-hrIgG1 is mediated by FcγRI, modulated via
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FcγRII, and enhanced by the presence of P-selectin on the platelet membrane.

L66 ANSWER 2 OF 4 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN DUPLICATE 1

2003098792 EMBASE Anti-HPA-1a-mediated platelet phagocytosis by monocytes in vitro and its inhibition by Fc gamma receptor (FcγR) reactive reagents.

Wiener, E., Dr. (correspondence); Abeyakoon, O.; Benchetrit, G.; Lyall, M.. Dept. of Haematology, St. Mary's, Imp. Coll. Fac. of Med., London, United Kingdom. e.wiener@ic.ac.uk. Keler, T.. Medarex Inc., Annandale, NJ, United States. Wiener, E., Dr. (correspondence); Rodeck, C.H.. Dept. of Haematology, Imperial College Faculty of Medicine, Hammersmith Hospital, DuCane Rd, London W12 0NN, United Kingdom. e.wiener@ic.ac.uk.

European Journal of Haematology Vol. 70, No. 2, pp. 67-74 1 Feb 2003. Refs: 42.

ISSN: 0902-4441. CODEN: EJHAEC

Pub. Country: United Kingdom. Language: English. Summary Language: English.

Entered STN: 20030325. Last Updated on STN: 20030325

AB The study was undertaken to delineate mechanisms of platelet destruction by phagocytosis during fetal/neonatal alloimmune thrombocytopenia (FAIT/NAIT) because of maternal antibodies against human platelet antigen 1a (HPA-1a). By employing a platelet phagocytosis assay based on the ORPEGEN flow cytometric bacterial phagocytosis test, we measured monocyte ingestion of platelets mediated by anti-HPA-1a antibodies. Moreover, we tested, as potential therapeutic agents, FcγTR reactive reagents, for their inhibition of this process. Four of six anti-HPA-1a sera tested mediated phagocytosis of HPA-1a-positive platelets in a concentration-dependent manner. Monocyte ingestion of platelets was almost completely inhibited by cytochalasin D. No anti-HPA-1a-mediated phagocytosis was observed with anti-HPA-1a -negative platelets. The humanised anti-FcγRI monoclonal antibody H22 at concentrations 1-100 μg/ml, completely inhibited anti-HPA-1a-mediated phagocytosis as did similar concentrations of ivIg. By contrast, a mouse monoclonal anti-FcγRII (IV.3, Fab) at 10 μg/ml caused little or no suppression of platelet phagocytosis mediated by two anti-HPA-1 sera. Furthermore, the addition of anti-FcγRII (10 μg/ml) to sub-optimal concentrations of H22 did not significantly increase the inhibitory effect of the latter compound. Monomeric IgG (0.1-10 μg/ml) failed to suppress anti-HPA-1 mediated platelet ingestion by the phagocytes, as did anti-FcγRIII. To our knowledge this is a rare example of an assay that measures platelet phagocytosis in vitro. The results suggest that FcγRI plays a major role in anti-HPA-1a-mediated platelet phagocytosis by monocytes while FcγRIIa, is of little or minor importance only. Moreover, the findings indicate the use of H22 as an alternative to interavenous Ig (ivIg) in the management of FAIT/NAIT.

L66 ANSWER 3 OF 4 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN

2002415405 EMBASE Human platelet antigen-1a antibodies induce the release of the chemokine RANTES from human platelets.

Dettke, M., Dr. (correspondence); Dreer, M.; Hocker, P.; Panzer, S.. Department for Blood Group Serology and Transfusion Medicine, AKH Wien, University of Vienna, Vienna, Austria. markus.dettke@univie.ac.at. Dettke, M., Dr. (correspondence). Department for Blood Group Serology and Transfusion Medicine, AKH Wien, Wahringer Gurtel 18-20, A-1090 Vienna, Austria. markus.dettke@univie.ac.at. Dettke, M., Dr. (correspondence). Department for Blood Group Serology, AKH Wien, Wahringer Gurtel 18-20,

A-1090 Vienna, Austria. markus.dettke@univie.ac.at.
Vox Sanguinis Vol. 81, No. 3, pp. 199-203 Oct 2001.
Refs: 29.
ISSN: 0042-9007. CODEN: VOSAAD
Pub. Country: United Kingdom. Language: English. Summary Language: English.
Entered STN: 20021205. Last Updated on STN: 20021205
AB Background and Objective: Binding of human platelet antigen-1a (HPA-1a)-specific antibodies to target platelets can trigger platelet activation and mediator release. Here we tested the effect of HPA-1a antibody-containing sera on platelet release of the chemokine RANTES (regulated on activation, normal, T-cell expressed, and presumably secreted) in vitro. Patients and Methods: HPA-1a-containing sera obtained from 11 mothers delivered of an infant with neonatal alloimmune thrombocytopenia (NAIT) and from six patients with post-transfusion purpura (PTP) were incubated with HPA-1a/a target platelets. Antibody-induced release of soluble RANTES was determined by enzyme-linked immunosorbent assay (ELISA). Results: A significant release of soluble RANTES was induced by four out of the 17 sera. Two out of the four reactive sera were obtained from mothers who were delivered of a baby with NAIT and the remaining two sera were from patients with PTP. Chemokine release was specific for binding of anti-HPA-1a to the platelet membrane, as none of the reactive sera induced the release of soluble RANTES when incubated with HPA-1b/b platelets. The blockade of platelet-expressed Fc gamma receptor type II (FcγRII) inhibited anti-HPA-1a-mediated RANTES release when incubated with the reactive sera of patients with NAIT, but not when platelets were incubated with sera of patients with PTP. Conclusion: Our findings suggest that anti-HPA-1a antibody-induced release of platelet-derived RANTES can play a role in adverse reactions in alloimmunized patients.

L66 ANSWER 4 OF 4 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN
2000434845 EMBASE HPA-1a-mediated platelet interaction with monocytes in vitro: Involvement of Fcγ receptor (FcγR) classes and inhibition by humanised monoclonal anti-FcγRI H22.
Wiener, E., Dr. (correspondence); Mawas, F.; Coates, P.; Hossain, A.K.; Perry, M.; Snachall, S.; Deb, P.; Rodeck, C.H.; Keler, T.. Department of Haematology, Imperial College School of Medicine, St. Mary's Campus, Norfolk Place, London W2 1PG, United Kingdom. e..wiener@ic.ac.uk.
European Journal of Haematology Vol. 65, No. 6, pp. 399-406 2000.
Refs: 38.
ISSN: 0902-4441. CODEN: EJHAEC
Pub. Country: Denmark. Language: English. Summary Language: English.
Entered STN: 20010111. Last Updated on STN: 20010111
AB To gain insight into mechanisms of platelet destruction and its possible inhibition during fetal/neonatal alloimmune thrombocytopenia (FAIT/NAIT) the binding to monocytes (Mo) of anti-HPA-1a-sensitised platelets, the initial step in IgG-mediated destruction by effector cells, was evaluated in an in vitro assay. Neonatal Mo were compared with adult Mo as effectors in the assay. Moreover, the potential involvement of Fcγ receptor (FcγR) classes during platelet destruction in the disease was tested by using FcγR class-specific reagents as inhibitors of the binding reaction. Neonatal Mo were 37% less active than adult Mo in their interaction with anti-HPA-1a-sensitised platelets ($p<0.05$). The FcγRI-specific reagents human monomeric IgG and humanised anti-FcγRI monoclonal H22 caused virtually complete inhibition of platelet binding to Mo. When compared to an intravenous immunoglobulin preparation the inhibitory activity of H22 was 10-100 x greater than that of the latter compound.

Monoclonal anti-FcγRII IV.3 and anti-FcγRIII 3G8 decreased platelet binding by 70% and 64%, respectively, but only the anti-FcγRII inhibition was statistically significant ($p<0.001$). Finally, anti-HPA-1a-sensitised platelets bound to 131H- but not to 131R- FcγRIIa transfected 3T6 mouse fibroblasts ($p<0.01$), in an anti-HPA-1a -concentration-dependent manner. The results suggest that FcγRI and FcγRIIa may be involved in anti-HPA-1a -mediated platelet destruction by mononuclear phagocytes during FAIT/NAIT. Moreover, the much greater potency of monoclonal H22 than of intravenous immunoglobulin as an inhibitor of anti-HPA-1a -mediated Mo-platelet interaction, might render it superior to the latter agent in the maternal therapy of the disorder.

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=> s anti-VAP-1
L67         62 ANTI-VAP-1

=> s l67 and CH2 domain
L68          0 L67 AND CH2 DOMAIN

=> s l67 and complement
L69          0 L67 AND COMPLEMENT

=> s l67 and Fc receptor
L70          2 L67 AND FC RECEPTOR

=> dup remove l70
PROCESSING COMPLETED FOR L70
L71           1 DUP REMOVE L70 (1 DUPLICATE REMOVED)

=> d l71 cbib abs

L71  ANSWER 1 OF 1  BIOSIS  COPYRIGHT (c) 2008 The Thomson Corporation  on STN
     DUPLICATE 1
2006:100021  Document No.: PREV200600094717. Function-blocking antibodies to
     human vascular adhesion protein-1: A potential anti-inflammatory therapy.
     Kirton, Christopher M.; Laukkanen, Marja-Leena; Nieminen, Antti; Merinen,
     Marika; Stolen, Craig M.; Armour, Kathryn; Smith, David J.; Salmi, Marko;
     Jalkanen, Sirpa; Clark, Michael R. [Reprint Author]. Univ Cambridge, Dept
     Pathol, Div Immunol, Tennis Court Rd, Cambridge CB2 1QP, UK.
     mrc7@cam.ac.uk. European Journal of Immunology, (NOV 2005) Vol. 35, No.
     11, pp. 3119-3130.
     CODEN: EJIMAF. ISSN: 0014-2980. Language: English.
AB   Human vascular adhesion protein-1 (VAP-1) is a homodimeric 170-kDa
     sialoglycoprotein that is expressed on the surface of endothelial cells
     and functions as a semicarbazide-sensitive amine oxidase and as an
     adhesion molecule. Blockade of VAP-1 has been shown to reduce leukocyte
     adhesion and transmigration in in vivo and in vitro models, suggesting
     that VAP-1 is a potential target for anti-inflammatory therapy. In this
     study we have constructed mouse-human chimeric antibodies by genetic
     engineering in order to circumvent the potential problems involved in
     using murine antibodies in man. Our chimeric anti-VAP
     -1 antibodies, which were designed to lack Fc-dependent effector
     functions, bound specifically to cell surface-expressed recombinant human
     VAP-1 and recognized VAP-1 in different cell types in tonsil.
     Furthermore, the chimeric antibodies prevented leukocyte adhesion and
     transmigration in vitro and in vivo. Hence, these chimeric antibodies
     have the potential to be used as a new anti-inflammatory therapy.
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=> s anti-alpha3
L72         101 ANTI-ALPHA3

=> s l72 and Fc function
L73           0 L72 AND FC FUNCTION

=> s l72 and substitution
L74           2 L72 AND SUBSTITUTION

=> s l74 and CH2 domain
L75           0 L74 AND CH2 DOMAIN

=> s l72 and CH2 domain
L76           0 L72 AND CH2 DOMAIN

=> s anti-NC1
L77          79 ANTI-NC1

=> dup remove l77
PROCESSING COMPLETED FOR L77
L78           31 DUP REMOVE L77 (48 DUPLICATES REMOVED)

=> s l78 and complement
L79           0 L78 AND COMPLEMENT

=> s l78 and pd<19980508
   1 FILES SEARCHED...
   4 FILES SEARCHED...
L80          19 L78 AND PD<19980508

=> d l80 1-19 cbib abs

L80  ANSWER 1 OF 19     MEDLINE on STN
96272575.    PubMed ID: 8688494.   Induction of Goodpasture antibodies to
     noncollagenous domain (NC1) of type IV collagen in mice by idiotypic
     manipulation. Shoenfeld Y; Gilburd B; Hojnik M; Damianovich M; Hacham S;
     Kopolovic Y; Polak-Charcon P; Goldberg I; Afek A; Hun-Chi L; +. (Research
     Unit of Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Israel. )
     Human antibodies and hybridomas, (1995) Vol. 6, No. 4, pp.
     122-8.  Journal code: 9014461. ISSN: 0956-960X. Pub. country: United
     States. Language: English.
AB   The characteristic pathogenic autoantibodies in Goodpasture's syndrome
     (GPS) are directed to the noncollagenous domain (NC1) of basement membrane
     type IV collagen.  To examine whether immunization with anti-
     NC1 antibodies could lead to GPS-like pathology, naive BALB/c mice
     were immunized intradermally with a mouse IgG anti-NC1
     monoclonal antibody or IgG serum fraction derived from patients with GPS.
     Mice immunized with normal mouse or human IgG and nonimmunized mice served
     as controls.  Anti-NC1 antibodies of IgG isotype were
     detected in the sera of mice injected with anti-NC1
     antibodies, but not in the sera of control mice.  The presence of
     circulating anti-NC1 antibodies coincided in some of
     the mice erythrocyturia or proteinuria and pathological changes in the
     kidneys.  No pathologic alterations were seen in the control mice.  The
     results show that specific idiotypic manipulation can induce anti
     -NC1 antibodies and pathological changes resembling human GPS.

L80  ANSWER 2 OF 19     MEDLINE on STN
96080350.    PubMed ID: 7586732.   Absence of anti-idiotypic antibodies in IVIG
     preparations to autoantibodies of rare autoimmune diseases. Krause I;
     Hacham S; Gilburd B; Damianovitch M; Blank M; Shoenfeld Y. (Department of
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Medicine B, Sheba Medical Center, Tel-Hashomer, Israel.) Clinical immunology and immunopathology, (1995 Dec) Vol. 77, No. 3, pp. 229-35. Journal code: 0356637. ISSN: 0090-1229. Pub. country: United States. Language: English.

AB Intravenous immunoglobulins (IVIG) were found to contain anti-idiotypic antibodies against autoantibodies of various autoimmune diseases. We examined commercial IVIG preparations, from three different manufactures, for the presence of autoantibodies and anti-idiotypic antibodies of two rare autoimmune diseases--primary biliary cirrhosis [anti-pyruvate dehydrogenase (PDH) antibodies] and Goodpasture's syndrome (anti -NC1 antibodies). We used ELISA studies as well as immunoblotting and anti-PDH enzyme activity for the detection of anti-PDH antibodies. ELISA and immunofluorescence studies were used for the detection of anti-NC1 antibodies. The presence of anti-idiotypic activity against anti-PDH fragments on Sepharose-bound IVIG [F(ab)2]. Anti-anti-NC1 activity was evaluated employing inhibition ELISA and immunofluorescence studies. The commercial IVIG preparations that were examined did not contain anti-PDH or anti-NC1 antibodies nor anti-idiotypic activity against these autoantibodies. We conclude that commercial IVIG may lack anti-idiotypic activity against rare autoantibodies.

L80 ANSWER 3 OF 19 MEDLINE on STN

95286260. PubMed ID: 7768595. Evidence for the presence of collagenous domains in Candida albicans cell surface proteins. Sepulveda P; Murgui A; Lopez-Ribot J L; Casanova M; Timoneda J; Martinez J P. (Departamento de Microbiologia, Facultad de Farmacia, Universitat de Valencia, Spain.) Infection and immunity, (1995 Jun) Vol. 63, No. 6, pp. 2173-9. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Rabbit polyclonal antibodies (PAbs) directed towards the amino-terminal cysteine-rich 7S domain (PAb anti-7S), the major internal collagenous domain (PAb anti-type IV), and the C-terminal noncollagenous region (PAb anti-NC1) of the type IV collagen molecule were probed by indirect immunofluorescence against Candida albicans blastoconidia and germinated blastoconidia. Most nongerminating cells and mother blastoconidia from which germ tubes originated showed strong fluorescence when PAb anti-7S was used, whereas with PAb anti-type IV, fluorescence was found almost exclusively on the surface of filamentous forms. A patched fluorescent pattern rather than a homogenous confluent fluorescence was observed in all cases. No fluorescent cells were observed with PAb anti-NC1. By Western immunoblotting, PAb anti-type IV cross-reacted primarily with a polypeptide of 37 kDa present in wall extracts obtained from intact cells of both growth forms by treatment with beta-mercaptoethanol, whereas PAb anti-7S recognized a major 58-kDa antigen also present in both extracts, along with some other high-molecular-mass (> 106-kDa) polydisperse species present only in the material released from blastoconidia with beta-mercaptoethanol. No reactive bands were observed when PAb anti-NC1 was used as a probe in Western immunoblotting experiments. The sensitivities or resistances to collagenase digestion of the different polypeptides that cross-reacted with PAbs anti-type IV and anti-7S suggest the existence of cell wall components in C. albicans that contain epitopes that mimic the collagenous domains of the type IV collagen molecule.

L80 ANSWER 4 OF 19 MEDLINE on STN

95089300. PubMed ID: 7527877. Characterization of anti-GBM antibodies involved in Goodpasture's syndrome. Hellmark T; Johansson C; Wieslander J. (Department of Nephrology, University Hospital, Lund, Sweden.) Kidney international, (1994 Sep) Vol. 46, No. 3, pp. 823-9. Journal code: 0323470. ISSN: 0085-2538. Pub. country: United States. Language:

English.

AB Goodpasture's syndrome is a life threatening autoimmune kidney disease. The patients have autoantibodies to the glomerular basement membrane, which are specific for the C-terminal domain of type IV collagen (NC1). The major antigen has been localized to the alpha 3 (IV)-chain. We have investigated sera from 44 patients with anti-NC1 antibodies. The quantity of antibodies to four different alpha(IV)-chains of type IV collagen was measured with direct ELISA. We used affinity chromatography to separate the antibodies and their specificities were studied with ELISA. The results show that about 1% of the patients total IgG are anti-NC1 antibodies and that 90% of these antibodies are specific for the alpha 3(IV)-chain. Antibodies to the other alpha(IV)-chains were found in 80% of the patients. Furthermore, affinity purified anti-alpha 3(IV) antibodies from one patient were inhibited by antibodies from the other patients, from 4 to 72%. The antibodies, from 39 of the patients, were inhibited by a monoclonal antibody against the alpha 3(IV)-chain. The results indicate that patients with Goodpasture's syndrome can have antibodies to most of the alpha(IV)-chains, while the majority of anti-NC1 antibodies are restricted to the alpha 3(IV)-chain. Moreover the number of epitopes seems to be limited and the majority of the antibodies from most patients are against one single epitope on the alpha 3(IV)-chain.

L80 ANSWER 5 OF 19 MEDLINE on STN

93102622. PubMed ID: 1466151. Production of anti-NC1 antibody by affected male dogs with X-linked hereditary nephritis: a probe for assessing the NC1 domain of collagen type IV in dogs and humans with hereditary nephritis. Thorner P S; Baumal R; Valli V E; Mahuran D; Marrano P M; Jacobs R. (Department of Pathology, Hospital for Sick Children, Toronto, Ontario, Canada.) Virchows Archiv. A, Pathological anatomy and histopathology, (1992) Vol. 421, No. 6, pp. 467-75. Journal code: 8302198. ISSN: 0174-7398. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Some patients with hereditary nephritis (HN) who have received a renal transplant have been shown to form antibody with specificity for the NC1 domain of collagen type IV, a major constituent of glomerular basement membranes (GBM). We attempted to duplicate this phenomenon in a family of dogs with X-linked HN, a model for human X-linked HN, by immunizing affected male dogs with normal dog NC1 domain. A collagenase digest was prepared from normal dog GBM, the NC1 domain was separated into dimer (approximately 50 kDa) and monomer (24 kDa and 26 kDa) components by SDS-PAGE, and injected into two affected male dogs. Antisera obtained from both dogs contained antibody which reacted with the NC1 domain of dog and human GBM by a plate-binding radioimmunoassay, bound to the dimer and 26 kDa monomer bands by Western blotting, and staining dog and human GBM by immunofluorescence (IF). The affected male dog antiserum reacted equally by radioimmunoassay with the NC1 domain isolated from GBM of unaffected, affected male, and carrier female dogs in the family with X-linked HN, and bound by Western blotting to dimers and the 26 kDa monomer band of the NC1 domain of GBM in each group of dogs. However, the affected male dog antiserum differentiated these dogs by IF; it produced global staining of GBM of unaffected dogs, failed to stain GBM of affected male dogs, and produced segmental staining of GBM of carrier female dogs. Absorption of the affected male dog antiserum with normal dog NC1 domain eliminated the staining of dog GBM by IF, whereas staining persisted after absorption with affected male dog NC1 domain. The abnormal staining patterns of GBM seen by IF in the affected male and carrier female dogs and the results of the absorption studies imply an abnormality of one or more determinants in the 26 kDa monomer band of the NC1 domain of their GBM. Amino acid sequencing of this band identified the alpha 1(IV) chain of collagen type IV, a finding that has implications for the pathogenesis

of canine X-linked HN. Absent and segmental staining respectively were also seen by IF in GBM of a male and female patient with HN, using the affected male dog antiserum. Thus, the results obtained in affected male and carrier female dogs with X-linked HN may also be relevant to patients with this disease.

L80 ANSWER 6 OF 19 MEDLINE on STN
93027896. PubMed ID: 1409187. Autoimmunity and glomerulonephritis. Saxena R; Johansson C; Bygren P; Wieslander J. (Department of Nephrology, University Hospital of Lund, Sweden.) Postgraduate medical journal, (1992 Apr) Vol. 68, No. 798, pp. 242-50. Ref: 83. Journal code: 0234135. ISSN: 0032-5473. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Autoimmunity is now unequivocally regarded as the predominant pathogenic process underlying most forms of primary and secondary glomerulonephritis in humans. Most of the investigations so far have been focused upon humoral mechanisms. Consequently, the role of cell-mediated immunity in nephritis is still incompletely understood. Nonetheless, as a result of contemporary studies, a number of previously unidentified auto-antibodies in association with glomerulonephritis have been discovered. However, apart from anti-NC1 antibodies in the classical Goodpasture syndrome, the exact role of these auto-antibodies in the pathogenesis of glomerulonephritis yet remains undefined. This fact, however, does not undermine the relevance of exploring these auto-antibodies. They have been of immense help in sub-classifying glomerulonephritis previously thought homogeneous (Figure 3). Besides, analysis of auto-antibodies has assisted tremendously in the early diagnosis of rapidly progressive glomerulonephritis. This, in turn, has aided in early commencement of therapy thus contributing to regression in morbidity and mortality resulting from these disorders. Moreover, investigation of these auto-antibodies is of enormous value for future studies aimed at understanding the pathogenic mechanisms involved in glomerulonephritis.

L80 ANSWER 7 OF 19 MEDLINE on STN
92273747. PubMed ID: 1317224. Antineutrophil-cytoplasmic antibodies and antiglomerular basement membrane antibodies in Goodpasture's syndrome and in Wegener's granulomatosis. Weber M F; Andrassy K; Pullig O; Koderisch J; Netzer K. (Department of Internal Medicine-Nephrology, University of Erlangen-Nurnberg, Germany.) Journal of the American Society of Nephrology : JASN, (1992 Jan) Vol. 2, No. 7, pp. 1227-34. Journal code: 9013836. ISSN: 1046-6673. Pub. country: United States. Language: English.
AB Antiglomerular basement membrane (anti-GBM) diseases-including Goodpasture's (GP) syndrome-and Wegener's granulomatosis (WG) are systemic diseases, which may be diagnosed by means of circulating autoantibodies. Possible overlap syndromes may exist; however, they remain imperfectly defined. We analyzed sera from 31 patients with WG and from 23 patients with anti-GBM disease. All underwent biopsy. Anti-cytoplasmic antibodies (ANCA) were demonstrated by indirect immunofluorescence (IIF); a perinuclear (P-ANCA) or diffuse-cytoplasmic (C-ANCA) staining was discerned. In addition, myeloperoxidase (MPO) antibodies (P-ANCA) and protein 3 (SP3) antibodies (C-ANCA) were analyzed by specific ELISA systems. Anti-GBM antibodies (anti-NC1 antibodies) were detected by ELISA and immunoblotting; the globular domain NC1 of collagen IV was employed as antigen. All 31 WG patients, as defined by clinical and histological criteria, showed ANCA by IIF. Twenty-nine of 31 showed a C-ANCA pattern; all were also positive for SP3 antibodies by ELISA. Three of 31 WG patients were P-ANCA positive by IIF and also had anti-MPO antibodies by ELISA. In one of these patients, SP3 antibodies were additionally found by IIF and by ELISA (double positive). No patient

with WG had anti-NC1 antibodies. All 23 serum samples from patients with GP syndrome (N = 19) or anti-GBM glomerulonephritis (N = 4) had anti-NC1 antibodies. In seven of these patients, low titers of anti-MPO antibodies were detected by ELISA; however, the IIF for ANCA was negative. None of these seven patients had extraglomerular vasculities. In addition, the clinical prognosis of these patients was similar to that of those patients who lacked these antibodies.(ABSTRACT TRUNCATED AT 250 WORDS)

L80 ANSWER 8 OF 19 MEDLINE on STN
92130598. PubMed ID: 1734140. [Autoantibodies as serological markers in the diagnosis of glomerulonephritis]. Autoantikroppar som serologiska markorer i diagnostiken av glomerulonefrit. Saxena R; Johansson C; Bygren P; Wieslander J. (Samtliga vid njurmedicinska sektionen, Lasarettet i Lund.) Lakartidningen, (1992 Jan 15) Vol. 89, No. 3, pp. 117-20. Journal code: 0027707. ISSN: 0023-7205. Pub. country: Sweden. Language: Swedish.

AB Autoimmunity is now regarded as the unequivocally predominant pathogenic process underlying most forms of primary and secondary glomerulonephritis in humans, and a number of autoantibodies occurring in conjunction with glomerulonephritis have been discovered. However, apart from the anti-NC1 antibodies occurring in classic Goodpasture's syndrome, the exact pathogenic role of these autoantibodies in human glomerulonephritis remains to be established, though this in no way diminishes the importance of their study. They have been of enormous value in subclassifying glomerulonephritis, previously thought to be a homogeneous entity. Autoantibody analysis has become a vital aid in the early diagnosis of rapidly progressive glomerulonephritis, which in turn has enabled treatment to be started early, thus contributing to a decline in the morbidity and mortality resulting from these disorders. Moreover, investigation of these autoantibodies will be of immense value in future studies focused on the pathogenic mechanisms involved in glomerulonephritis.

L80 ANSWER 9 OF 19 MEDLINE on STN
91343156. PubMed ID: 1652114. Circulating autoantibodies in patients with extracapillary glomerulonephritis. Saxena R; Bygren P; Rasmussen N; Wieslander J. (Department of Nephrology, University Hospital of Lund, Sweden.) Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association, (1991) Vol. 6, No. 6, pp. 389-97. Journal code: 8706402. ISSN: 0931-0509. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Circulating autoantibodies, namely c-ANCA, MPO-ANCA, anti-Goodpasture (anti-NC1), and anti-entactin antibodies were analysed in sera from 82 consecutive patients with crescentic involvement of more than 50% glomeruli in renal biopsy specimens. Sixty-eight (approximately 83%) patients possessed one or more of these autoantibodies. About two-thirds of all patients had ANCA (c-ANCA, MPO-ANCA or both). Most of the remaining positive patients had anti-NC1 antibodies. Very few patients had anti-entactin antibodies, thereby suggesting a poor association of these antibodies with extracapillary glomerulonephritis (ECGN). Thus two different categories of patients, one possessing ANCA and the other anti-NC1 antibodies, could be recognised. Patients with anti-NC1 antibodies were characterised by linear immune deposits along the glomerular basement membrane and the clinical outcome was invariably grim. On the other hand, despite no significant difference in renal morphology from patients with anti-NC1 antibodies, the disease in patients with ANCA, in general, had a milder course. Among patients with ANCA, those with c-ANCA mainly had systemic small-vessel vasculitis with widespread

systemic manifestations, whereas most patients with renal restricted primary ECGN with non-linear immune deposits possessed MPO-ANCA. Furthermore, patients with c-ANCA had a more severe disease than those with MPO-ANCA. These observations indicate that a continuous spectrum of diseases exists between idiopathic small-vessel vasculitides and primary non-linear ECGN. Our study also demonstrates that the presence of auto-antibodies is a dominant feature of severe ECGN and that the type of immunological injury is more important than the extent of crescentic involvement of glomeruli in determining the course of illness in patients with ECGN.

L80 ANSWER 10 OF 19 MEDLINE on STN
91185417. PubMed ID: 2010469. The NC1 domain of type IV collagen promotes axonal growth in sympathetic neurons through interaction with the alpha 1 beta 1 integrin. Lein P J; Higgins D; Turner D C; Flier L A; Terranova V P. (Department of Pharmacology and Therapeutics, School of Medicine, State University of New York, Buffalo 14214.) The Journal of cell biology, (1991 Apr) Vol. 113, No. 2, pp. 417-28. Journal code: 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.
AB We have examined the effects of collagen IV on the morphological development of embryonic rat sympathetic neurons in vitro. In short-term (less than or equal to 24 h) culture, collagen IV accelerated process outgrowth, causing increases in the number of neurites and total neuritic length. Analysis of proteolytic fragments of collagen IV indicated that the NC1 domain was nearly as active as the intact molecule in stimulating process outgrowth; in contrast, the 7S domain and triple helix-rich fragments of collagen IV were inactive. Moreover, anti-NC1 antiserum inhibited neuritic outgrowth on collagen IV by 79%. In long-term (up to 28 d) cultures, neurons chronically exposed to collagen IV maintained a single axon but failed to form dendrites. Thus, the NC1 domain of collagen IV can alter neuronal development by selectively stimulating axonal growth. Comparison of collagen IV's effects to those of laminin revealed that these molecules exert quantitatively different effects on the rate of initial axon growth and the number of axons extended by sympathetic neurons. Moreover, neuritic outgrowth on collagen IV, but not laminin, was blocked by cycloheximide. We also observed differences in the receptors mediating the neurite-promoting activity of these proteins. Two different antisera that recognize beta 1 integrins each blocked neuritic outgrowth on both collagen IV and laminin; however, an mAb (3A3) specific for the alpha 1 beta 1 integrin inhibited collagen IV but not laminin-induced process growth in cultures of both sympathetic and dorsal root neurons. These data suggest that immunologically distinct integrins mediate the response of peripheral neurons to collagen IV and laminin.

L80 ANSWER 11 OF 19 MEDLINE on STN
90142524. PubMed ID: 2105558. A study by immunofluorescence microscopy of the NC1 domain of collagen type IV in glomerular basement membranes of two patients with hereditary nephritis. Thorner P S; Baumal R; Eddy A; Marrano P M. (Department of Pathology, Hospital for Sick Children, Toronto, Ontario, Canada.) Virchows Archiv. A, Pathological anatomy and histopathology, (1990) Vol. 416, No. 3, pp. 205-12. Journal code: 8302198. ISSN: 0174-7398. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.
AB The NC1 domain of the collagen type IV molecule, the major component of glomerular basement membranes (GBM), consists of dimers and 24 kilodalton (K), 26 K and 28 K monomers in man, and contains the Goodpasture antigen. Serum obtained from patients with Goodpasture's syndrome has been reported not to stain GBM of most male and some female patients with hereditary nephritis (HN) by immunofluorescence (IF) microscopy. In the present study, GBM seen on the renal biopsies of 2 patients (one male and one

female) with HN were examined by IF to ascertain whether NC1 monomers were detectable. Three reagents were used: a plasmapheresis fluid (PPF) obtained from a patient who was treated for anti-GBM nephritis (human anti-GBM PPF); a commercial rabbit antibody against human NC1; and a rabbit antibody raised by us against dog NC1, which cross-reacted with human NC1. All 3 reagents detected NC1 determinants in GBM of normal human kidney by IF and reacted with human NC1 by a plate-binding radioimmunoassay (RIA). The human anti-GBM PPF bound to 28 K and 26 K monomer components of NC1 by Western blotting, the rabbit anti-human NC1 antibody bound to 26 K and 24 K monomers, while the rabbit anti-dog NC1 antibody bound only to the 26 K monomer. By IF, the human anti-GBM PPF did not stain GBM of the male patient with HN, but produced segmental staining of GBM (i.e., some GBM stained, while others did not) of the female patient. In contrast, the rabbit anti-NC1 antibodies produced global staining by IF of GBM of both patients. The absence of staining (i.e., global or segmental) seen with the human anti-GBM PPF implied that the 26 K and 28 K monomers of NC1 were either absent from GBM, or were present but altered structurally, leading to a diminution in their immunological reactivity. However, the positive staining observed with the rabbit anti-NC1 antibodies implied that the 26 K monomer was actually present in GBM. Hence, we postulate that the 26 K monomer of NC1 in GBM was structurally altered, and that the 28 K monomer was either absent, or present but altered. These findings suggest that there is an abnormality of more than one monomer of NC1 in GBM of patients with HN.

L80 ANSWER 12 OF 19 MEDLINE on STN
89244887. PubMed ID: 2719074. The NC1 domain of collagen type IV in neonatal dog glomerular basement membranes. Significance in Samoyed hereditary glomerulopathy. Thorner P; Baumal R; Binnington A; Valli V E; Marrano P; Clarke H. (Department of Pathology, Hospital for Sick Children, University of Toronto, Ontario, Canada.) The American journal of pathology, (1989 May) Vol. 134, No. 5, pp. 1047-54. Journal code: 0370502. ISSN: 0002-9440. Pub. country: United States. Language: English.

AB Patients with hereditary nephritis (HN) present with renal disease after infancy, suggesting that the lesion of glomerular basement membranes (GBM) may not be congenital. Therefore, the NC1 domain of collagen type IV in normal neonatal dog GBM was compared with NC1 in normal adult GBM by SDS-PAGE and Western blotting, using two anti-NC1 antibodies. Similar results were obtained, indicating that the NC1 domain is present and immunoreactive in the neonatal period. Next, serial renal biopsies were performed on a family of Samoyed dogs with hereditary glomerulopathy (SHG), an animal model of HN, and assessed by immunofluorescence. One of the anti-NC1 antibodies produced global staining of GBM in unaffected dogs, and global/segmental staining in carrier females; however, no staining was seen in affected males as early as the neonatal period. Electron microscopy (EM) failed to demonstrate any lesion of GBM in neonatal dogs. Thus, in SHG, and presumably in human HN, the abnormality in the NC1 domain is congenital, and precedes the changes seen by EM in GBM.

L80 ANSWER 13 OF 19 MEDLINE on STN
89215678. PubMed ID: 2708945. Radioimmunoassay for immunoreactive non-collagenous domain of type IV collagen (NC1) in serum: normal pregnancy and preeclampsia. Bieglmayer C; Hofer G. (2nd Dept. of Obstetrics and Gynecology, University of Vienna.) Journal of clinical chemistry and clinical biochemistry. Zeitschrift fur klinische Chemie und klinische Biochemie, (1989 Mar) Vol. 27, No. 3, pp. 163-7. Journal code: 7701860. ISSN: 0340-076X. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB The use of a magnetic separation agent in a modified radioimmunoassay for the non-collagenous cross-linked region (NC1) of basement membrane collagen is described. The rabbit anti-NC1 serum employed in the assay revealed a binding behaviour similar to that reported recently (Mark et al. (1985) Eur. J. Biochem. 146, 555-562; Schuppan et al. (1986) J. Clin. Invest. 78, 241-248) with respect to the integrity of NC1-disulphide bridges, affinity to NC1 subunits and lack of reactivity with other determinants of type IV collagen. Immunoreactive serum NC1, which increased towards the end of gestation, showed a broad peak during the second trimester of pregnancy. In preeclampsia, immunoreactive serum-NC1 was slightly elevated.

L80 ANSWER 14 OF 19 MEDLINE on STN
89209420. PubMed ID: 2706812. Characterization of the NC1 domain of collagen type IV in glomerular basement membranes (GBM) and of antibodies to GBM in a patient with anti-GBM nephritis. Thorner P S; Baumal R; Eddy A; Marrano P. (Department of Pathology, Hospital for Sick Children, Toronto, Ontario, Canada.) Clinical nephrology, (1989 Mar) Vol. 31, No. 3, pp. 160-8. Journal code: 0364441. ISSN: 0301-0430. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.
AB Anti-glomerular basement membrane (GBM) nephritis is associated with production of antibodies to the Goodpasture antigen (GPA) component of the NC1 domain of collagen type IV. We studied a patient with anti-GBM nephritis with regard to 1) reactivity of the anti-GBM antibodies in his serum, plasmapheresis fluid (PPF), and an eluate prepared from GBM of his nephrectomy specimen, and 2) electrophoretic and immunologic properties of the NC1 domain extracted by collagenase digestion from GBM of his nephrectomy specimen. Antibodies to different NC1 determinants in serum, PPF and eluate were detected by immunofluorescence of glomerular capillaries of normal kidney. In addition, the antibody in PPF, but not in the eluate, reacted strongly in a plate-binding radioimmunoassay with NC1 domain extracted from normal human GBM, and bound by Western blotting to both dimer (46 kD and 49 kD) and monomer (24 kD, 26 kD and 28 kD) components of the NC1 domain. Analysis of the NC1 domain in the patient's GBM by SDS-PAGE showed a number of abnormalities, including an absence of monomer bands. Moreover, there was diminished reactivity of the patient's NC1 domain by the radioimmunoassay and Western blotting, using his PPF and a rabbit anti-NC1 antiserum. These findings indicated that there were different types of antibodies to NC1 domain in PPF and eluate, associated with an abnormal NC1 domain in GBM. These results have allowed us to speculate on the pathogenesis of anti-GBM nephritis in this patient.

L80 ANSWER 15 OF 19 MEDLINE on STN
88299705. PubMed ID: 2841385. Involvement of nucleoli and dense bodies in the intranuclear distribution of some capsid polypeptides in cells infected with herpes simplex virus type 1. Puvion-Dutilleul F; Cebrian J. (Groupe de Laboratoires, l'Institut de Recherches Scientifiques sur le Cancer, CNRS, Villejuif, France.) Journal of ultrastructure and molecular structure research, (1988 Mar) Vol. 98, No. 3, pp. 229-42. Journal code: 8612238. ISSN: 0889-1605. Pub. country: United States. Language: English.
AB The distribution of capsid proteins induced by herpes simplex virus type 1 infection was determined at the ultrastructural level. The antiserum A to total capsid proteins and the anti-NC1 and NC2 sera, all labeled with gold particles, decorated the entire thickness of both empty capsids and nucleocapsids filled with viral DNA. On the other hand, an antibody to NC3,4 protein produced a heavy labeling concentrated almost entirely along the internal surface of empty capsids, whereas full capsids were not labeled. DNase digestion of "full" capsids did not restore anti-NC3,4 protein binding at this site. Published biochemical data

concerning viral protein distribution in capsids are conflicting, but if NC3,4 protein is present in full capsids, we suggest that new binding forces between capsid proteins occurred at the time of insertion of viral DNA which might conceal the relevant antigenic sites of NC3,4 proteins. Capsid proteins were abundantly present in the viral nucleoplasm and in most constituents of the infected cells particularly some nucleoli and some but not all dense bodies. However, whereas anti-NC1 serum labeled nucleoli but not dense bodies, both anti-NC2 and anti-NC3,4 sera stained only dense bodies but not nucleoli. Inhibition of replication of viral DNA which entered the cell during the infective period did not inhibit the production of capsid proteins. Inhibition of protein synthesis in late infected cells did not alter the distribution of capsid proteins.

L80 ANSWER 16 OF 19 MEDLINE on STN
87057696. PubMed ID: 3782304. The role of the main noncollagenous domain (NC1) in type IV collagen self-assembly. Tsilibary E C; Charonis A S. The Journal of cell biology, (1986 Dec) Vol. 103, No. 6 Pt 1, pp. 2467-73. Journal code: 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.
AB Type IV collagen incubated at elevated temperatures in physiologic buffers self-associates (a) via its carboxy-terminal (NC1) domain, (b) via its amino-terminal (7S) domain, and (c) laterally; and it forms a network. When examined with the technique of rotary shadowing, isolated domain NC1 was found to bind along the length of type IV collagen to four distinct sites located at intervals of approximately 100 nm each. The same 100-nm distance was observed in domain NC1 of intact type IV collagen bound along the length of the collagen molecules during initial steps of network formation and in complete networks. The presence of anti-NC1 Fab fragments in type IV collagen solutions inhibited lateral association and network formation in rotary shadow images. During the process of self-association type IV collagen develops turbidity; addition of isolated domain NC1 inhibited the development of turbidity in a concentration-dependent manner. These findings indicate that domain NC1 of type IV collagen plays an important role in the process of self-association and suggest that alterations in the structure of NC1 may be partially responsible for impaired functions of basement membranes in certain pathological conditions.

L80 ANSWER 17 OF 19 MEDLINE on STN
86309188. PubMed ID: 3528661. Globular domain of basement membrane collagen induces autoimmune pulmonary lesions in mice resembling human Goodpasture disease. Wick G; Von der Mark H; Dietrich H; Timpl R. Laboratory investigation; a journal of technical methods and pathology, (1986 Sep) Vol. 55, No. 3, pp. 308-17. Journal code: 0376617. ISSN: 0023-6837. Pub. country: United States. Language: English.
AB A distinct circulating antibody response could be evoked in C57BL mice after immunization with the globular domain NC1 of basement membrane collagen IV obtained from the mouse Engelbreth-Holm-Swarm tumor when injected together with complete Freund's adjuvant. The antibodies reacted with various subunits of NC1, did not cross-react with other basement membrane proteins, and exhibited a tissue reactivity restricted to certain basement membranes. Tissue-bound antibodies could be detected by direct immunofluorescence and were distributed together with C3 in a linear pattern along glomerular and alveolar basement membranes. Pathological changes were mainly observed in lung and kidney and consisted of inflammatory infiltrates and massive hemorrhages with strong granulomatous fibrotic development in the lung. Kidney alterations were comparably weaker and of focal nature. A nephrotoxic serum model showed rapid binding of rabbit antibodies against mouse NC1 to lung, liver, and kidney basement membranes which was followed several weeks later by an autologous

phase with anti-rabbit IgG antibodies bound to basement membranes as immune complexes. There was no fibrotic response but hemorrhagic and inflammatory lung and kidney changes similar to those after active immunization were observed after passive transfer. The experimental NC1 autoimmune model has several features such as anti-NC1 response, tissue restriction, lung hemorrhages, and glomerulonephritis in common with patients suffering from Goodpasture disease. The development of lung fibrosis appears to be unique for the animal model.

L80 ANSWER 18 OF 19 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN

1989082151 EMBASE Abnormalities in the NC1 domain of collagen type IV in GBM in canine hereditary nephritis.

Thorner, P.; Baumal, R.; Valli, V.E.O.; Mahuran, D.; McInnes, R.; Marrano, P.. Department of Pathology, Hospital for Sick Children, Toronto, Ont. M5G 1X8, Canada.

Kidney International Vol. 35, No. 3, pp. 843-850 1989.

ISSN: 0085-2538. CODEN: KDYIA5

Pub. Country: United States. Language: English. Summary Language: English.

Entered STN: 911212. Last Updated on STN: 911212

AB Samoyed hereditary glomerulopathy (SHG) in dogs serves as a model for human X-linked hereditary nephritis (HN). We previously showed that glomerular capillaries of affected males did not stain by immunofluorescence (IF) using serum from a patient with Goodpasture's syndrome. Our goal in the present study was to determine whether the NC1 domain of the collagen type IV molecule, which contains Goodpasture antigen (GPA), could be demonstrated in these dogs, and to assess its immunological reactivity. By SDS-PAGE, NC1 in collagenase digests of glomerular basement membranes (GBM) of unaffected and carrier female dogs in the family with SHG showed 24 kilodalton (kD), 26 kD and 28 kD monomer, and 46 kD and 47 kD dimer components, but the 24 kD monomer was diminished in the affected males. By IF, a rabbit antibody to NC1 stained glomerular capillaries of unaffected, affected male, and carrier female dogs. In contrast, a human anti-GBM plasmapheresis fluid (PPF) stained glomerular capillaries of only the unaffected and carrier female dogs. By RIA, both antibodies reacted strongly with NC1 in collagenase digests of GBM of the unaffected and carrier female dogs, but showed reduced reactivity with NC1 of affected males. By Western blotting, both antibodies bound to dimers and 24 kD and 26 kD monomers of the NC1 domain in collagenase digests of GBM of unaffected and carrier female dogs. However, in affected males, the rabbit anti-NC1 antibody did not bind to the 24 kD monomer, while the human anti-GBM PPF showed weak binding to the 24 kD and 26 kD monomers. Hence, although the NC1 domain could be detected in GBM of affected male dogs, a reduced amount of the 24 kD monomer was present and, as well, the 26 kD monomer possessed altered immunological reactivity. These 2 monomers are known to be derived from separate autosomal gene products in man. Hence, our studies raise the possibility that, in SHG and X-linked HN, the underlying defect may involve a protein which is coded on the X chromosome and is involved in modifying the collagen type IV molecule.

L80 ANSWER 19 OF 19 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

1996:102443 The Genuine Article (R) Number: TT001. Pathogenicity of anti-basement membrane (NC1) antibodies: An experimental Goodpasture's syndrome. Shoenfeld Y (Reprint). CHAIM SHEBA MED CTR, DEPT MED B, AUTOIMMUNE DIS RES UNIT, IL-52621 TEL HASHOMER, ISRAEL (Reprint); TEL AVIV UNIV, SACKLER FAC MED, IL-69978 TEL AVIV, ISRAEL. ISRAEL JOURNAL OF MEDICAL SCIENCES (JAN 1996) Vol. 32, No. 1, pp. 29-31. ISSN: 0021-2180. Publisher: ISRAEL JOURNAL MED SCIENCES, 2 ETZEL ST, FRENCH HILL, JERUSALEM 97853, ISRAEL. Language: English.

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=> s anti-CD3
L81      31787 ANTI-CD3

=> s l81 and CH2 domain
L82          7 L81 AND CH2 DOMAIN

=> dup remove l82
PROCESSING COMPLETED FOR L82
L83          3 DUP REMOVE L82 (4 DUPLICATES REMOVED)

=> d l83 1-3 cbib abs

L83  ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2008 ACS on STN
2007:44867   Document No. 146:227655  Method for designing mini-antibody
     (minibody) against CD3 without mitogen activity.  Lu, Ming; Feng, Jiannan;
     Shen, Beifen; Huang, Ying; Gu, Xin; Li, Yan (Beijing Tianguangshi
     Biological Technology Co., Ltd., Peop. Rep. China).  Faming Zhuanli
     Shenqing Gongkai Shuomingshu CN 1891716 A  20070110, 23pp.  (Chinese).
     CODEN: CNXXEV.  APPLICATION: CN 2010-82945 20050708.
AB   The title method is characterized in deleting CH1 domain and CH2
     domain containing Fc receptor binding sites so as to remove mitogen
     activity of antibody, and stabilizing the divalent structure of the
     antibody by using CH3 domain and corresponding hinge region.  The
     invention relates to the variable region gene of the title small mol.
     antibody against CD3.  The invention also relates to method for extending
     the hinge region of human IgG so as to increase its flexibility.  The
     antibody can be used for preventing and treating acute rejection in organ
     transplantation, and also treating autoimmune diseases, such as
     insulin-dependent diabetes mellitus, amyotrophic lateral sclerosis, etc.

L83  ANSWER 2 OF 3     MEDLINE on STN                    DUPLICATE 1
2004617353.    PubMed ID: 15589476.   Anti-CD3 antibodies
     OKT3 and hOKT3gamma1(Ala-Ala) induce proliferation of T cells but impair
     expansion of alloreactive T cells; aspecifc T cell proliferation induced
     by anti-CD3 antibodies correlates with impaired
     expansion of alloreactive T cells. Popma Sicco H; Griswold Don E; Li Li.
     (Centocor, Inc. 200 Great Valley Parkway, Malvern, PA 19355, USA..
     spopma@cntus.jnj.com) . International immunopharmacology, (2005 Jan) Vol.
     5, No. 1, pp. 155-62.  Journal code: 100965259. ISSN: 1567-5769. Pub.
     country: Netherlands. Language: English.
AB   OKT3, a mouse anti-human CD3 monoclonal antibody (mAb), has been used for
     decades to reverse acute transplant rejection.  A humanized OKT3 with two
     alanine replacements on the CH2 domain,
     hOKT3gamma1(Ala-Ala), was generated to reduce side effects of OKT3.  This
     study reports the effects of OKT3 and hOKT3gamma1(Ala-Ala) on responder T
     cells in mixed leukocyte cultures (MLC).  T cells were purified from
     peripheral blood mononuclear cells (PBMC) and labeled with CFSE before
     culture with irradiated allogeneic PBMC in a MLC.  Multiparameter flow
     cytometric analysis of MLC demonstrated logarithmic expansion of T cells
     with a memory phenotype.  Treatment with either OKT3 or
     OKT3gamma1(Ala-Ala) prevented the logarithmic expansion of these cells.
     Instead, both OKT3 and hOKT3gamma1(Ala-Ala) induced nonspecific expansion
     of T cells with both naive and memory phenotype.  The proliferating T
     cells in OKT3 or hOKT3gamma1(Ala-Ala) treated cultures expressed low
     levels of CD25, and showed a diminished Granzyme B expression compared to
     that of the untreated MLC, suggesting that these cells may not be fully
     functional.  An array of cytokines in the MLC supernatant was analyzed,
     and IL-5 and IL-13 were significantly reduced by OKT3 or
     hOKT3gamma1(Ala-Ala) treatment.  These results demonstrated that OKT3 and
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hOKT3gamma1(Ala-Ala) have similar effects on T cells in MLC. Both antibodies inhibited the logarithmic expansion of allo-reactive T cells, probably through inducing suboptimal proliferation of specific and non-specific T cells. This in vitro study provided one possible mechanism of the therapeutic effects of OKT3 and hOKT3gamma1(Ala-Ala) antibodies.

L83 ANSWER 3 OF 3 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

2003:531395 The Genuine Article (R) Number: 691BG. Limited role of charge matching in the interaction of human immunoglobulin A with the immunoglobulin A Fc receptor (Fc alpha RI) CD89. Pleass R J; Dehal P K; Lewis M J; Woof J M (Reprint). Univ Dundee, Sch Med, Ninewells Hosp, Dept Mol & Cellular Pathol, Dundee DD1 9SY, Scotland (Reprint); Univ Dundee, Sch Med, Dept Mol & Cellular Pathol, Dundee, Scotland. IMMUNOLOGY (JUL 2003) Vol. 109, No. 3, pp. 331-335. ISSN: 0019-2805. Publisher: BLACKWELL PUBLISHING LTD, 9600 GARSINGTON RD, OXFORD OX4 2DG, OXON, ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Human immunoglobulin A (IgA) mediates protective effector mechanisms through interaction with specific cellular Fc receptors (FcalphaRI). Two IgA Fc interdomain loops (Leu257-Leu258 in the CH2 domain and Pro440-Phe443 in the CH3 domain) have previously been identified as critical for binding to FcalphaRI. On the receptor, the interaction site for IgA has been localized to the EC1 domain. The essential FcalphaRI residues involved are Tyr35, Tyr81 and Arg82, with contributions also from Arg52 and to a lesser extent from His85 and Tyr86. The basic nature of the side chains of some of the receptor residues implicated in ligand binding suggested that charge matching might play some role in the interaction. To address this possibility, we have generated five IgA1 mutants with point substitutions in acidic residues lying close to the putative interaction site and assessed their abilities to bind FcalphaRI on human neutrophils. Mutants E254A, E254L and E437A displayed affinities for FcalphaRI comparable to that of wild-type IgA1, while mutants D255A and D255V had only slightly reduced affinities for the receptor. Therefore, electrostatic interactions appear unlikely to play a significant role in the IgA-FcalphaRI interaction. Moreover, the lack of effect of mutations in residues adjacent to those previously implicated in binding, reaffirms the importance of the interdomain loops in FcalphaRI binding.

=> s anti-Der pI
L84 1 ANTI-DER PI

=> d l84 cbib abs

L84 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2008 ACS on STN

1993:621130 Document No. 119:221130 Original Reference No. 119:39321a,39324a Antigen detection apparatus. Mitchell, Edmund Bruce; Shattock, Alan Gaylard; Joyce, Patrick Joseph (University College Dublin, Ire.). PCT Int. Appl. WO 9318404 A1 19930916, 64 pp. DESIGNATED STATES: W: AU, CA, JP, NZ, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1993-IE8 19930304. PRIORITY: IE 1992-700 19920305.

AB A receptacle for collection of an antigen, e.g. a dust mite allergen, dispersed in a motile fluid in the environment is formed of a porous material, typically nylon mesh with pore size 15-60 μm, coated with antibody which retains the antigen coming into contact therewith. The receptacle has a removable holder for locating it in the pipe of a vacuum cleaner. The receptacle can be used in an immunoassay for detection or determination of the antigen. Thus, a tubular air filter of 40-μm nylon mesh

was coated with a monoclonal antibody to Der pI antigen (cysteine protease of Dermatophagoides pteronyssinus) and mounted in a vacuum cleaner pipe so that all the intake air passed through the filter. After vacuuming selected sites, the filter was removed, extracted with phosphate-buffered saline solution containing Tween 20 to transfer allergen to the nylon mesh, washed, and incubated successively with an anti-Der pI IgG-peroxidase conjugate and H2O2/tetramethylbenzidine-2HCl solution for spectrophotometric determination

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=> s anti-lamiin
L85          0 ANTI-LAMIIN

=> s anti-laminin
L86       1676 ANTI-LAMININ

=> s l86 and CH2 domain
L87          0 L86 AND CH2 DOMAIN

=> s l86 and reduced complement
L88          0 L86 AND REDUCED COMPLEMENT

=> s l86 and complement
L89         49 L86 AND COMPLEMENT

=> s l89 and substitution
L90          0 L89 AND SUBSTITUTION

=> s l89 and CH2
L91          0 L89 AND CH2

=> s l89 and Eu system
L92          0 L89 AND EU SYSTEM

=> s anti-lutheran
L93         19 ANTI-LUTHERAN

=> s l93 and CH2 domain
L94          0 L93 AND CH2 DOMAIN

=> dup remove l93
PROCESSING COMPLETED FOR L93
L95         10 DUP REMOVE L93 (9 DUPLICATES REMOVED)

=> s l95 and pd<19980508
   2 FILES SEARCHED...
L96          7 L95 AND PD<19980508

=> d l96 1-7 cbib abs

L96  ANSWER 1 OF 7     MEDLINE on STN
2006397521.    PubMed ID: 16810817.   A Further Case of Anti-
     Lutheran Immunization, with Some Studies on its Capacity for Human
     Sensitization. Mainwaring U R. (National Blood Transfusion Service,
     Regional Serological Laboratory (Oxford Region). ) Journal of clinical
     pathology, (1948 Nov) Vol. 1, No. 5, pp. 292-4.  Journal code:
     0376601. ISSN: 0021-9746. Pub. country: England: United Kingdom. Language:
     English.

L96  ANSWER 2 OF 7     MEDLINE on STN
59055202.    PubMed ID: 13625656.   Inhibition of anti-Rh and anti-
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Lutheran sera by ribonucleic acid derivatives. HACKEL E; SMOLKER R E; FENSKE S A. Vox sanguinis, (1958 Dec) Vol. 3, No. 6, pp. 402-8. Journal code: 0413606. ISSN: 0042-9007. Language: English.

L96 ANSWER 3 OF 7 MEDLINE on STN
54095632. PubMed ID: 13175487. Hypersplenism with anti-Lutheran antibody following transfusion. SHAW S; MOURANT A E; IKIN E W. Lancet, (1954 Jul 24) Vol. 267, No. 6830, pp. 170-1. Journal code: 2985213R. ISSN: 0140-6736. Language: English.

L96 ANSWER 4 OF 7 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN
1982203352 EMBASE Management of pregnancies with rare blood types. Biale, Y.; Dvilansky, A.. Div. Obstet. Gynecol., Soroka Med. Cent., Beer-Sheba, Israel.
Acta Obstetricia et Gynecologica Scandinavica Vol. 61, No. 3, pp. 219-221 1982.
ISSN: 0001-6349. CODEN: AOGSAE
Pub. Country: Sweden. Language: English.
Entered STN: 911209. Last Updated on STN: 911209
AB Pregnant women with antibodies to high incidence blood group antigens should be diagnosed as early as possible and red blood cells should be stored frozen for future possible needs. Either autologous, compatible siblings' or unrelated individuals' red blood cells should be frozen. The application of such a program is described in three pregnant women wih exceedingly rare antibodies to high incidence blood group antigens (anti-Lutheran(b), and PP(1)P(k), and anti c with blood type B CCddee D(u) negative).

L96 ANSWER 5 OF 7 EMBASE COPYRIGHT (c) 2008 Elsevier B.V. All rights reserved on STN
1979139138 EMBASE Autologous blood transfusions and pregnancy. Sandler, S.G.; Beyth, Y.; Laufer, N.; Levene, C.. Dept. Obstet. Gynecol. Hadassah-Hebrew Univ. Med. Cent., Jerusalem, Israel.
Obstetrics and Gynecology Vol. 53, No. 3 SUPPL., pp. 62S-66S 1979.
ISSN: 0029-7844. CODEN: OBGNAS
Pub. Country: United States. Language: English.
AB The application of autologous and frozen red blood cell (rbc) programs is described for 3 pregnant women with antibodies to high-incidence blood group antigens (anti-Lutheran(b), anti-Cellano, anti-Vel). The cases illustrate how readily available supplies or rare blood types can be maintained throughout pregnancy using autologous and frozen RBC techniques, including selective predeposit, 'family-sharing', and intensive phlebotomy with fluid replacement. The RBC phenotypes described in this paper are exceedingly rare since they occur in only 0.1-0.001% of random donors. However, the principles of autologous blood transfusions are universal and they can be applied to the general problems of blood group incompatibility in pregnancy.

L96 ANSWER 6 OF 7 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
1949:1297 Document No.: PREV19492300001310; BA23:1310. Two examples of sera containing the anti-d agglutinin predicted by Fisher and Race. HILL, JOSEPH M.; HABERMAN, SOL. Southwestern Med Coll., Dallas. NATURE [LONDON], (1948) Vol. 161, No. 4096, pp. 688-689.
Language: Unavailable.
AB Two iso-immunized mothers, each of genotype CDe/CDe, had small doses of cde/ cde cells. Serum agglutinated cells of types CDe/cde, cde/cde, and CDe/CDe, but none with only D when test-tube methods were used; Coombs' anti-human globulin gave positive results with all erythrocytes containing c and/or d. Tests ruled out correlation with A, B, [image], N, or P, nor

were these sera anit-Kell, anti-Lutheran, or
anti-Lewis. Hemolysis was equal with one c or one d; twice as much with
one of each. Specificity of 65.16% agreed closely with Fisher's
prediction, and was antithetical to that of anti-D serum. ABSTRACT
AUTHORS: R. Walker

L96 ANSWER 7 OF 7 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
1946:19157 Document No.: PREV19462000019312; BA20:19312. A serolo-gical and
genetical study of multiple antibodies formed in response to blood
transfusion by a patient with lupus erythe-matosus diffusus. Callender,
Sheila T.; Race, R. R.. ANN EUGENICS, (1946) Vol. 13, No. 2, pp.
102-117.
Language: Unavailable.
AB A patient suffering from lupus erythematosus diffusus has developed the
following antibodies in response to blood transfusions: 1) anti-c (St,
anti-Hr), a rare Rh agglutinin; 2) the "incomplete" form of this antibody;
3) anti-Lutheran which agglutinates the blood of 8% of
the English population rrespective of the ABO, MN, P or Rh groups; 4)
anti-Willis (anti-C w), a form of anti-Rh not previously recognized, which
has resulted in the extention of knowledge about Rh antigens and genes; 5)
anti-Levay, an antibody to another hitherto unrecognized antigen which is
inherited, but is apparently rare in the English population; 6) anti-iV,
an agglutinin only twice before identified in human serum. Clearly the
patient exhibits a remarkable pronencss to make iso-agglutinins in
response to the introduction of antigens which are ignored by most people.
The discovery of a new gene, allelic to C and c, further confirms the
necessity for the analytic separation of the Rh genes as foreseen by
Fisher. ABSTRACT AUTHORS: Authors

=> s (armour k?/au or clark m?/au or williamson l?/au)
L97 15119 (ARMOUR K?/AU OR CLARK M?/AU OR WILLIAMSON L?/AU)

=> s l97 and immunoglobulin
L98 484 L97 AND IMMUNOGLOBULIN

=> s l98 and modified
L99 10 L98 AND MODIFIED

=> s l99 and complement mediated lysis
L100 1 L99 AND COMPLEMENT MEDIATED LYSIS

=> d l100 cbib abs

L100 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2008 ACS on STN
1993:579181 Document No. 119:179181 Original Reference No. 119:32031a,32034a
Humanized antibodies having modified allotypic determinants.
Clark, Michael Ronald (Lynxvale Ltd., UK). PCT Int. Appl. WO
9216562 A1 19921001, 56 pp. DESIGNATED STATES: W: CA, JP, US; RW: AT,
BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE. (English). CODEN:
PIXXD2. APPLICATION: WO 1992-GB445 19920312. PRIORITY: GB 1991-5245
19910312.
AB Humanized antibodies lacking ≥1 allotypic determinant in the constant
region of their Ig heavy chains are prepared by substituting an
allotypic determinant from the corresponding position of an equivalent constant
region of an Ig of different isotype. The synthesized
Igs have reduced allotypic differences as compared to the
wild-type Ig. Humanized rat CAMPATH-1H IgG1 monoclonal antibody
was further modified by site-directed mutagenesis. The site
corresponding to the G1m(1) allele was converted to the corresponding
sequence found in the other subclasses for IgG (Asp-Glu-Leu to Glu-Glu-Met

at positions 356-358 in the CH3 domain). Addnl., the lysine residue responsible for the G1m(17) allotypic determinant at position 214 in the CH1 domain was converted to an arginine residue as found in IgG3 and IgG4 isotypes to make mutant 1. A further mutant, mutant 2, was made by replacing the critical arginine residue 214 associated with the G1m(3) allele site of mutant 1 with a threonine residue, to produce a heavy chain which is the equivalent of IgG2. Genes encoding mutants 1 and 2 were incorporated into expression vectors containing the CAMPATH-1H V-region gene and expressed together with the CAMPATH-1H light chain. An ELISA was performed to verify that an IgG1-type antibody was produced by the mutants. The mutants were active in autologous complement-mediated lysis of human peripheral blood lymphocytes and in antibody-dependent cell-mediated cytotoxicity assays. In a conventional allotyping experiment, mutant 1 typed as allotype G1m(3), while mutant 2 failed to allotype for any of the IgG1 allotype markers.

=> s l98 and CH2 domain
L101 21 L98 AND CH2 DOMAIN

=> dup remove l101
PROCESSING COMPLETED FOR L101
L102 6 DUP REMOVE L101 (15 DUPLICATES REMOVED)

=> d l102 1-6 cbib abs

L102 ANSWER 1 OF 6 MEDLINE on STN DUPLICATE 1
2003519950. PubMed ID: 14597161. Differential binding to human FcgammaRIIa and FcgammaRIIb receptors by human IgG wildtype and mutant antibodies. Armour Kathryn L; van de Winkel Jan G J; Williamson Lorna M; Clark Mike R. (Department of Pathology, Division of Immunology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK.) Molecular immunology, (2003 Dec) Vol. 40, No. 9, pp. 585-93. Journal code: 7905289. ISSN: 0161-5890. Pub. country: England: United Kingdom. Language: English.

AB We are investigating the interactions of recombinant human IgG antibodies with Fc receptors to enable selection of a constant region giving minimal depletion of antigen-bearing cells. Eight variant constant regions were made by substituting motifs between human IgG subclasses in the lower hinge region and/or a specially close loop of the CH2 domain. Mutations in the lower hinge region were shown to eliminate FcgammaRI binding and monocyte activation [Eur. J. Immunol. 29 (1999) 2613]. Here, we detail interactions with FcgammaRIIa of the 131R and 131H allotypes and FcgammaRIIb. Lower hinge mutations caused large reductions in binding whereas modification of residues 327, 330 and 331 had less dramatic effects. However, like the wildtype IgG subclass binding hierarchies, the effect of the mutations varied between different receptors. We identified IgG1 variants which react with the activating receptor, FcgammaRIIa, at least 10-fold less efficiently than wildtype IgG1 but whose binding to the inhibitory receptor, FcgammaRIIb, is only four-fold reduced. Manipulation of interactions with FcgammaRIIb separately from those with activating receptors provides potential for designing antibodies with novel and effective combinations of attributes. In addition, insight is gained into the evolution of functional differences in human IgG subclasses.

L102 ANSWER 2 OF 6 MEDLINE on STN DUPLICATE 2
2002438623. PubMed ID: 12196122. The contrasting IgG-binding interactions of human and herpes simplex virus Fc receptors. Armour K L; Atherton A; Williamson L M; Clark M R. (Department of Pathology, University of Cambridge, Cambridge, UK..

kla22@mole.bio.cam.ac.uk) . Biochemical Society transactions, (2002 Aug) Vol. 30, No. 4, pp. 495-500. Ref: 37. Journal code: 7506897. ISSN: 0300-5127. Pub. country: England: United Kingdom. Language: English.

AB A virally encoded, high-affinity Fc receptor (FcR) is found on herpes simplex virus type 1 (HSV-1) particles and infected cells where its binding of non-immune IgG protects cells from host-mediated lysis. Whilst mutation or aglycosylation of the IgG CH2 domain reduced binding to human FcR, the interaction with HSV-1 FcR was not affected. However, the HSV-1 FcR, unlike human FcR, discriminates between human IgG1 allotypes, being sensitive to changes at positions 214 (CH1) and 356/358 (CH3), away from its proposed binding site at the CH2-CH3 interface. The biological consequences are not known but this is the first evidence of a major functional difference between IgG1 allotypes.

L102 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2008 ACS on STN

1999:736770 Document No. 131:350263 Chimeric proteins containing IgG Fc fragments which do not trigger complement mediated lysis. Armour, Kathryn Lesley; Clark, Michael Ronald; Williamson, Lorna McLeod (Cambridge University Technical Services Limited, UK). PCT Int. Appl. WO 9958572 A1 19991118, 81 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB1441 19990507. PRIORITY: GB 1998-9951 19980508.

AB The authors disclose recombinant polypeptides comprising: (i) a binding domain capable of binding a target mol., and (ii) an effector domain having an amino acid sequence substantially homologous to all or part of a constant domain of a human Ig heavy chain. These chimeric proteins are capable of binding the target mol. without triggering significant complement dependent lysis, or cell mediated destruction of the target and, via the effector domain, remain capable of specifically binding FcRn and/or FcγRIIb. These effector domains are derived from two or more human Ig heavy chain CH2 domains. The binding domain of the chimeric proteins may be derived from antibodies, enzymes, hormones, receptors, and cytokines etc.

L102 ANSWER 4 OF 6 MEDLINE on STN DUPLICATE 3

1999388014. PubMed ID: 10458776. Recombinant human IgG molecules lacking Fcgamma receptor I binding and monocyte triggering activities. Armour K L; Clark M R; Hadley A G; Williamson L M. (Division of Immunology Department of Pathology, University of Cambridge, Cambridge, GB.) European journal of immunology, (1999 Aug) Vol. 29, No. 8, pp. 2613-24. Journal code: 1273201. ISSN: 0014-2980. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Subclasses of human IgG have a range of activity levels with different effector systems but each triggers at least one mechanism of cell destruction. We are aiming to engineer non-destructive human IgG constant regions for therapeutic applications where depletion of cells bearing the target antigen is undesirable. The attributes required are a lack of killing via Fcgamma receptors (R) and complement but retention of neonatal FcR binding to maintain placental transport and the prolonged half-life of IgG. Eight variants of human IgG constant regions were made with anti-RhD and CD52 specificities. The mutations, in one or two key regions of the CH2 domain, were restricted to incorporation of motifs from other subclasses to minimize potential immunogenicity. IgG2 residues at positions 233 - 236, substituted into IgG1 and IgG4, reduced binding to FcgammaRI by 10(4)-fold and eliminated the human monocyte response to

antibody-sensitized red blood cells, resulting in antibodies which blocked the functions of active antibodies. If glycine 236, which is deleted in IgG2, was restored to the IgG1 and IgG4 mutants, low levels of activity were observed. Introduction of the IgG4 residues at positions 327, 330 and 331 of IgG1 and IgG2 had no effect on FcgammaRI binding but caused a small decrease in monocyte triggering.

L102 ANSWER 5 OF 6 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN

1995:111533 The Genuine Article (R) Number: QG208. INTERACTION OF HUMAN MONOCYTE FC-GAMMA RECEPTORS WITH RAT IGG2B - A NEW INDICATOR FOR THE FC-GAMMA-RIIA (R-H131) POLYMORPHISM. HAAGEN I A (Reprint); GEERARS A J G; CLARK M R; VANDEWINKEL J G J. UNIV UTRECHT HOSP, DEPT IMMUNOL F03821, POSTBOX 85500, 3508 GA UTRECHT, NETHERLANDS (Reprint); UNIV CAMBRIDGE, DEPT PATHOL, CAMBRIDGE, ENGLAND. JOURNAL OF IMMUNOLOGY (15 FEB 1995) Vol. 154, No. 4, pp. 1852-1860. ISSN: 0022-1767. Publisher: AMER ASSOC IMMUNOLOGISTS, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Rat mAbs receive considerable interest for immunologic intervention in man. The rat IgG2b isotype has previously been found to be optimally active both in vivo and in vitro. We found that both a rat IgG2b CD3 mAb and a monovalent hybrid rat IgG2b-mouse IgG1 bispecific Ab triggered T cell activation in PBMC. Inhibition analyses with mAb blocking different human IgG Fc receptors (Fc gamma R) showed a dimorphic pattern. In donors expressing an Fc gamma RIIa-R/R131 allotype (previously defined on the basis of interaction with mouse (m) IgG1 as ''high responder'') anti-Fc gamma RI mAb 197 inhibited rat IgG2b induced T cell mitogenesis almost completely. In Fc gamma RIIa-H/H131 (''low responder'' allotype) donors, however, both anti-Fc gamma RI mAb 197 and anti-Fc gamma RII mAb IV.3 were essential for optimal inhibition of mitogenesis. T cell proliferation experiments performed with the use of Fc gamma R-transfected fibroblasts as accessory cells showed the high affinity Fc gamma RIa (CD64) to interact with both rat IgG2b and rat IgG2b-m1gG1 hybrid CD3 mAb. The use of the two types of Fc gamma RIIa (CD32)-transfectants instead showed rat IgG2b CD3 mAb to interact solely with the IIa-H/H131 allotype. Interestingly, rat IgG2b-m1gG1 hybrid mAb did not interact effectively with this low affinity Fc gamma R. This suggests a requirement for only one rat IgG2b H chain for Fc gamma RIa-mediated binding, whereas two identical H chains seem to be necessary for proper interaction with Fc gamma RIIa. Ab-sensitized RBC-rosette experiments performed with the use of a rat IgG2b anti-NIP mAb confirmed the interaction pattern observed with rat CD3 mAb, supporting the phenomena to be isotype-, and not mAb-, dependent. These analyses point to a unique reactivity pattern for rat IgG2b Abs, interacting both with the high affinity Fc gamma RIa in all donors and Fc gamma RIIa of individuals expressing the IIa-H131 allotype.

L102 ANSWER 6 OF 6 MEDLINE on STN DUPLICATE 4

93238866. PubMed ID: 8477804. Structural motifs involved in human IgG antibody effector functions. Greenwood J; Clark M; Waldmann H. (Department of Pathology, University of Cambridge.) European journal of immunology, (1993 May) Vol. 23, No. 5, pp. 1098-104. Journal code: 1273201. ISSN: 0014-2980. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB A humanized IgG antibody to CAMPATH-1 antigen (CDw52) is known to be lympholytic both in vitro and in vivo. So as to improve therapeutic potency through protein engineering strategies, we wish to define the structural motifs underlying some of the documented differences in function between human (h) IgG1 and IgG4 forms of the antibody. By the creation of heavy chain domain-switch and intra-domain recombinant antibodies we have established an important role for the carboxy-terminal

half of the CH2 domain in determining differential behaviour in antibody-dependent cytotoxicity (ADCC) and in complement lysis. If this same region were necessary for the effector mechanisms that operate in vivo, then it might be possible to improve antibody effector functions by construction of novel antibodies that possess within the one molecule multiple copies of the crucial hinge-CH2 associated structures. Although our previous work suggested that the hIgG4 CAMPATH-1 antibody was ineffective at ADCC, we found this to be so only in some individuals. In others, IgG4, and indeed all the IgG subclasses were able to mediate ADCC. Overall, though, hIgG1 remains the best choice isotype for lytic therapy in vivo.

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